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Plant Biotechnology Institute

Annual Report 1987-1988



The National Research Council in Biotechnology

Canada's National Research Council (NRC) is a Crown-owned corporation for research and development established in 1916. NRC performs and promotes research and development for national economic and social benefits through an array of national laboratories, programs for transfer of new technology to industries, and scientific-technical information services.

Since 1924 the Council has maintained a strong program in biological sciences, and in 1983 was commissioned by the Government of Canada to lead the national effort in biotechnology. For this initiative the Council has integrated the programs of its Division of Biological Sciences (Ottawa) on leading-edge methods research with those of the NRC's new Biotechnology Research Institute (Montreal) which spearheads industrial innovation, and of the Plant Biotechnology Institute (Saskatoon). The present NRC biotechnology program involves more than five hundred core personnel.

The Plant Biotechnology Institute has an explicit mission: to develop new exploitable biological and biochemical methods for the control and genetic alteration of plant development, especially at the cellular and molecular levels.

This mission reflects NRC's purpose under the National Research Council Act, to "promote research (i) . . . on the utilization of the natural resources of Canada; (ii) with the object of . . . discovering processes and methods that may promote the expansion of existing or the development of new industries; (iii) . . . to improve conditions in agriculture".

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1987 - 88 Annual Report
Plant Biotechnology Institute
National Research Council of Canada

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Cover photo: *Pipette tips used to transfer buffer solutions*

1. Highlights of the Year 1987/88

The Government of Canada created the Plant Biotechnology Institute (PBI) in 1983, with the mandate to function as Canada's premiere institution for advanced research on biotechnologies for higher plants. Our operations since 1948 under the name "Prairie Regional Laboratory" had already established us as an international centre for plant cell technology and phytochemical research. With the change of name and mission came new resources and an opportunity to undertake a thrust of major dimensions to ensure that Canada will be a strong participant in the biotechnological revolution already upon us. Our mission is to produce world-class plant biotechnology for development in Canada.

This year the Institute successfully completed the first phase of that development thrust begun in 1983. This phase had three objectives: to strengthen our gene technology capabilities; to quicken our research projects' progress without sacrificing the scientific or technical quality of the outputs; and to develop more effective business and outreach activities.

The organization of the Institute into three research Laboratories -- for Plant Cell Technology, Plant Gene Technology and Biological Chemistry -- has proved ideal in marrying provision of facilities with performance of research projects. PBI now has strength in plant gene technology not surpassed in

Canada. Fourteen Ph.D. scientists and 12 technical officers are devoted entirely to such work, with a further 11 scientists and 14 technical officers engaged in closely allied projects. The Institute has been fortunate in having attracted new staff of the very highest calibre for this initiative. As this Annual Report details, our work has already proven its scientific merit and its economic relevance.

PBI's **research facilities** offer resources for specific techniques and a setting for advanced research by staff and guests. The Institute's **research program** consists of six projects based in one or another of our Laboratories but often involving external collaborators. These project groups were very productive in 1987/88. Among the highlights of the year:

- The **Crop Biotechnology** project, using cell selection methods recently developed at the Institute, has established cell and whole plant lines of winter wheats exhibiting a new record of survival at sub-zero temperatures. Some of this work is being carried out through a joint venture with the Saskatchewan Wheat Pool.

- **Conifer Biotechnology** at PBI showed substantial progress this year. We advanced our ability to grow conifer cells in artificial cultures and to regenerate plantlets, and in collaboration with University of Saskatchewan researchers we

achieved complete regeneration of embryos from protoplasts of white spruce. This has opened the door to genetic engineering approaches to conifer improvement, where our techniques for transformation of conifer cells have already furnished putative transgenic material.

- The **Plant Molecular Genetics** project developed a very effective new technique for insertion of foreign genes into rapeseed (*Brassica*) cells, with transformation frequency of 100% in regenerated plants. The group has gone on to use the technique for successful introduction of herbicide resistance genes into canola and other species.

- In the **Plant Productivity Genes** project, researchers identified gene-products in pea plants carrying the nodulating and non-nodulating alleles of a nodulating specific gene. They also proved that genetic variations in levels of the photosynthetic enzyme RUBISCO are correlated with synthesis of its small-subunit messenger RNA.

- In the **Bio-organic Chemistry** project, chemists discovered new synthetic hormones which show strong effects on plant growth. Evaluation of the agronomic potential of these compounds is continuing in 1988 with scientists from the University of Saskatchewan and with other collaborators. The project also initiated major new work on metabolic modifications in plants using DNA technology.

- The **Plant Cell Metabolism** project continued development of new plant cell bioreactors and studied their use in production of new pharmaceutical metabolites including sanguinarine. First successes were scored in gene expression regulation of metabolic pathways.

The winning of two President's Fund awards for innovative basic research in 1987, and another two awards in 1988 (four out of a total of eighteen awards given nationally) attested to the continuing high quality of science in PBI laboratories. As to

quantity, a record number of scientific papers from PBI authors appeared in research journals during 1987. In recognition of excellence, PBI obtained two more shared NSERC grants, along with awards for joint ventures with industry. On the technology side, the Institute ended the year with two new patents, four further applications in process, and ten technology development licenses offered. During the course of 1987/88, four major joint venture Agreements were signed -- with the University of Saskatchewan Crop Development Center, the Saskatchewan Wheat Pool, Vipont Research Laboratories, Inc., and Biotechnica Canada Inc. -- and a much larger number of smaller collaborations were underway.

The success of the Institute's research program, and the National Research Council's increasing role in technology transfer, have made "doing business" an important activity of PBI. Matters such as business plans, explicit patenting and licensing efforts, negotiated commercialization ventures with industrial partners, or written inter-organizational Agreements for long-term research, were rarely on our desks five years ago. Today they are never absent, and the Institute has a special unit dedicated to biotechnology development ventures.

The Guest Researcher program continued to expand through the year as more and more Canadian companies, universities and public agencies sent personnel to PBI for training and collaborative work. The nearly seventy guests contributed 37 person-years of research time and more than three quarters of a million dollars in salary equivalence to the Institute, a measure of the importance of this program to the participating organizations.

A larger and stronger research effort; closer industrial and university links; the prospect of dynamic growth ahead: these make the need for well-informed decision-making more and more important at PBI. In the autumn of 1987 a new advisory element came into being with the formation of the

PBI Board of Governors. This council brings twelve knowledgeable people from government, academia and the private sector together as strategic advisors in the Institute's forward planning process.

The year 1988/89 will be a time of continued productivity at the Plant Biotechnology Institute notwithstanding persisting financial constraints. Our doors are open to research collaborations with other public agencies, with university laboratories, and above all with private sector firms. We hope that this Annual Report will prove useful and informative to readers, and that it conveys some sense of the

excitement that today pervades the field of plant biotechnology.

Warren Steck

Dr. Warren Steck
Director of the Institute

F. Constabel

Dr. Fred Constabel
Assistant Director of the Institute



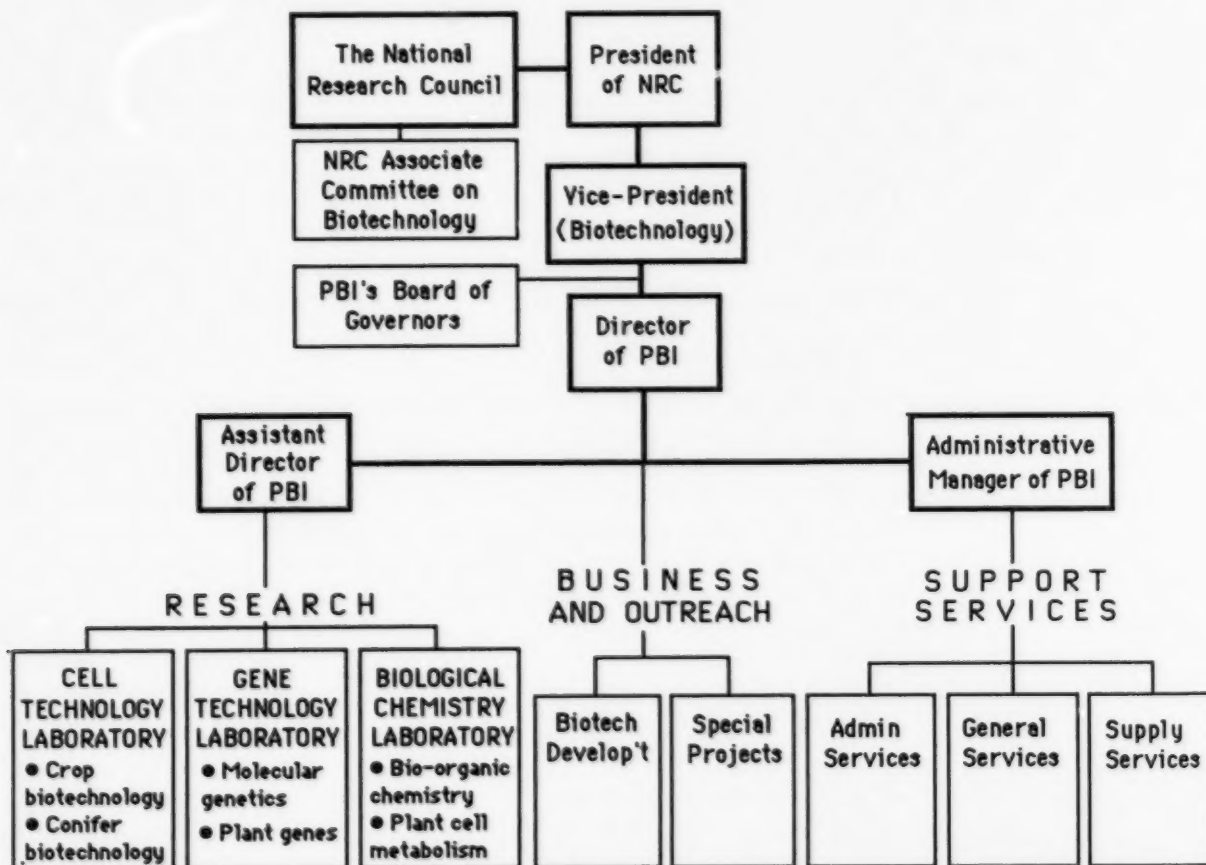
The Plant Biotechnology Institute of the National Research Council of Canada in Saskatoon.

2. Organization and Personnel of the Institute

2.1 Organization

The Plant Biotechnology Institute has the usual structure of a National Research Council laboratory

division, as shown in the chart, with research, services and business/outreach sections.



2.2 The Board of Governors

As part of the National Research Council's policy of having its national laboratories strategically advised by the sectors they serve, the PBI Board of Governors was created in 1987. Briefly, its terms of reference are to provide strategic planning advice and to comment on the annual resource requirements of the Institute.

The Chairman of the Board is Dr. Maurice BROSARD, Senior Vice-President (Laboratories) and Vice-President (Biotechnology), National Research Council of Canada.

Other members are:

Dr. Guy BELLEMARE, Full Professor, Faculty of Science, University of Laval, (Laval, Quebec)

Dr. Wallace BEVERSDORF, Chairman of the Dept of Crop Science, University of Guelph (Guelph, Ontario)*

Dr. Anthony FRENCH, Chief Executive Officer, Forintek Canada (Vancouver, British Columbia)

Dr. Leo KRISTJANSON, President, University of Saskatchewan (Saskatoon, Saskatchewan)

Dr. Maurice LALONDE, Professor of Biology, Laval University (Sainte-Foy, Québec)

Dr. Kelvin OGILVIE, Vice-President (Academic), Acadia University (Wolfville, Nova Scotia)

Dr. Arthur OLSON, Assistant Deputy Minister (Research) Agriculture Canada (Ottawa, Ontario)

Dr. Warren STECK, Director, Plant Biotechnology Institute, NRC Canada (Saskatoon, Saskatchewan)

Mr. Ted TURNER, President, Prairie Pools Inc. (Regina, Saskatchewan)

Mr. Gordon WELLS, Chief Executive Officer, Biotechnica Canada (Calgary, Alberta)

Dr. John WILLIAMS, Chairman of the Dept of Botany, University of Toronto (Toronto, Ontario)

The Board met twice during the fiscal year, on 18 November 1987 and on 10 March 1988. Both meetings were held in Saskatoon.

* at date of appointment was Vice-President, Allelix Agriculture (Mississauga, Ontario)

2.3 Staff, Affiliates and Guest Researchers in 1987/88

An asterisk (*) indicates a limited-term staff appointment. Some staff were not present for the entire year.

Director: Warren F. STECK, B.Eng. (McGill);
Ph.D. (Saskatchewan)

Assistant Director: F. CONSTABEL, Dr. rer. nat. (Gottingen), Dr. Habil. (Bonn)

Administrative Officer: Joan L. YORKE

Principal Research Officers:

W.G.W. KURZ, Ph.D. (Vienna)
E.W. UNDERHILL, B.S.P., M.Sc. (Saskatchewan);
Ph.D. (Rhode Island)

Senior Research Officers:

P.S.S. DAWSON, B.Sc., M.Sc. (Birmingham);
Ph.D. (Saskatchewan)
D.I. DUNSTAN, B.Sc., Ph.D.
(NE London Polytechnic)
K.N. KAO, B.S. (Chung-Hsing, China);
M.S.A. (Guelph); Ph.D. (Saskatchewan)
K.K. KARTHA, B.Sc. (Saugar); M.Sc. (Jawaharlal);
Ph.D. (New Delhi)
S.L. MacKENZIE, B.Sc., Ph.D. (Edinburgh)
J.D. MAHON, B.Sc. (Western Ontario);
M.Sc., Ph.D. (Queen's)
G.P. SLATER, B.Sc. (Aberdeen);
M.Sc. (Saskatchewan); Ph.D. (Queen's, Belfast)

Associate Research Officers:

S.R. ABRAMS, B.Sc. (Carleton); Ph.D. (Dalhousie)
J.J. BALSEVICH, B.Sc., M.Sc., Ph.D.
(British Columbia)
W. CROSBY, B.Sc. (British Columbia);
Ph.D. (Edinburgh)
A.J. CUTLER, B.Sc. (Wales), Ph.D. (Florida State)

F. GEORGES, M.Sc. (Brock); Ph.D. (Ottawa)
J.W.D. GROOTWASSINK, B.Sc., M.Sc.
(Wageningen); Ph.D. (Calgary)
S. HEMMINGSEN, B.Sc. (British Columbia);
Ph.D. (Queen's)
S.L.A. HOBBS, M.A. (Cambridge);
B.A., D. Phil. (Oxford)
M.S. LAPP, B.Sc., M.Sc. (York); Ph.D. (Alberta)
M. MAZUREK, B.S.A. (Saskatchewan)
L.M. NELSON, B.Sc. (Western Ontario);
Ph.D. (Calgary)
L.E. PELCHER, B.S. (Aquinas);
M.S., Ph.D. (Michigan State)
R.D. REICHERT, B.Sc., Ph.D. (Saskatchewan)
G. SELVARAJ, B.Sc. (Bangalore);
M.Sc., Ph.D. (Carleton)
R.T. TYLER, B.S.A., Ph.D. (Saskatchewan)

Assistant Research Officers:

V. DELUCA, B.Sc. (Concordia); M.Sc. (Queen's);
Ph.D. (Western Ontario)
M. SALEEM, B.Sc., M.Sc. (Pakistan);
M.Sc. (Laurentian); Ph.D. (Western Ontario)
J. TAYLOR, B.Sc. (Iowa State);
Ph.D. (Washington State)
E.W.T. TSANG, B.Sc., M.Sc., Ph.D.
(Saskatchewan)

Research Council Officers:

J. Sylvan KATZ*, B.Sc., M.Sc. (Saskatchewan)
T. Martin MALLARD

NSERC Fellows (Funded by PBI):

F. BEKKAOUI*, B.Sc. (Berkane);
M.Sc. (Tours, France); Ph.D. (Paris)
L. KLIMCZAK*, M.Sc., Ph.D. (Wurzburg)
L.A.K. NELSON*, B.Sc. (U.S.T.); Ph.D. (Alberta)
M.S.C. PEDRAS*; B.Sc., M.Sc. (Porto);
Ph.D. (Alberta)

Research Associates:

J.G. BOOTHE*, B.Sc., Ph.D. (Western Ontario)
C.P. CHANWAY*, B.Sc., B.S.A. (Manitoba);
M.Sc., Ph.D. (British Columbia)
S. CHEVADEJ*, B.Sc., M.Eng. (Chulalongkorn);
Dr. Res. Nat. (Heidelberg)
R. CHIBBAR*, B.Sc., M.Sc. (Meerut);
Ph.D. (Panjab); Ph.D. (Western Ontario)
A. GOOD*, B.Sc. (Trent); M.Sc. (Windsor);
Ph.D. (Ottawa)
A. HUSSAIN*, B.Sc. (Iraq); Ph.D. (Aston)
J.C. JAIN*, B.Sc. (Rajasthan); Ph.D. (Jodhpur)
E.J. KENDALL*, B.Sc., M.Sc. (Dalhousie);
Ph.D. (Guelph)
M.F. LeGAL*, D.E.A., Ph.D. (Nantes, France)
J.L.R. MARTEL*, B.Sc., M.Sc., Ph.D. (Laval)
W.J. NEWSTED*, B.Sc., M.Sc., Ph.D.
(Western Ontario)
N.L. PAIVA*, B.Sc. (Harvey Mudd); Ph.D. (M.I.T.)
K.A. PIVNICK*, B.Sc. (Brit. Col.); Ph.D. (Laval)
J. QURESHI*, B.Sc., M.Sc. (Karachi);
Ph.D. (Liverpool)
D.S. RAJU*, B.Sc., M.Sc., Ph.D. (Andhra)
I. ROEWER*, M.Sc., Ph.D. (Muenster)
D. TAYLOR*, B.Sc., Ph.D. (Carleton)
L.W. THOMSON*, B.Sc., M.Sc., Ph.D. (Alberta)
R.J. WESELAKE*, B.Sc., M.Sc., Ph.D. (Manitoba)

Visiting Research Officer

Alain DAVID*, B.Sc., M.Sc. (Bordeaux);
Ph.D. (Paris)

Laboratory Technical Officers

David BALISKI
Carla BARBER, B.Sc. (Saskatchewan)
Dennis BARTON, B.Sc. (Saskatchewan)
Mary BECKIE, B.Sc. (Saskatchewan)
Colleen BENNETT*, B.S.T. (K.I.A.A.S.)
Terry BETHUNE, B.Sc. (Saskatchewan)
Greg BISHOP, B.Sc. (Saskatchewan)
Cheryl BOCK, B.Sc. (Saskatchewan)
Karen CASWELL, B.S.A., M.Sc. (Saskatchewan)

K. Brock CHATSON, C.R.T. (S.A.I.T.)
Maxine COFFEY, B.L.T. (Regina)
Janet CONDIE, B.Sc. (Queen's)
Catherine DELONG, B.S.A., B.Sc. (Saskatchewan)
Maureen DENNIS, B.Sc. (Saskatchewan)
John DYCK*
Don DYMOND
Mike GIBLIN
J. HAMMERLINDL
Rozina HIRJI, B.Sc. (Makerere)
Lawrence HOGGE
Les HOFFMAN
David HORN
Connie KNAPP
Diana KNEESHAW, B.Sc. (Saskatchewan)
Allan KOLENOVSKY, B. Sc. (Saskatchewan)
Nick LEUNG, B.Sc. (Saskatchewan)
Mary LOEWEN, B.S.A. (Saskatchewan)
Cliff MALLARD
John MAGUS, B.Sc. (Saskatchewan)
Jana MALINEK, B.Sc., M.Sc. (Palacky)
Morris MICHAYLUK
Melanie OLSON*, B.Sc. (Saskatchewan)
Doug OLSON, B.S.T. (K.I.A.A.S.)
Kelth PAHL
Barry PANCHUK
Bob PAPISH, B.Sc. (Saskatchewan)
Sandra POLVI, B.S.T. (K.I.A.A.S.)
Darwin REED, B.Sc. (Saskatchewan)
Don SCHWAB
Angela SHAW, B.Sc. (Saskatchewan)
Jerry SHYLUK
Lee STEINHAEUER, B.A. (Saskatchewan)
Don TENASCHUK, B.Sc. (Saskatchewan)
Maureen WOOD*, B.S.T. (K.I.A.A.S.)

Administrative Services:

Cheryl BITZ
Rosemarie GALLAYS
Dawn LARSON
Kathy SEABROOK
Carol WALKER
Kathy WILSON

Supply Services:

Olga COATES
Jill HORN
Terry MAZURKEVICH
Jim McHARG
Irene PHILIPCHUK
Bill WILEY

Research Illustration:

Ed KNAPP

General Services:

Bill BRUMWELL
Armand FENDELET
Harry HOLFELD
Rick McDONALD
George REYNOLDS
Wayne STANKEWICH

Special Projects:

Lorraine BLASHILL

Summer Students* (1987)

Francis CHAN
Sophie GIRARD
Daniel GLAESKE
Thierry LEGROS
Madeleine LEVESQUE
Laurie LITWINSON
Chrystal McNABB
Adrienne NIKIFORUK
Marc PILON
William STIRTON
Howard VANZEGEREN
Michael WILSON

Co-op Student* (1987)

Michael WILSON

Affiliated Scientists of the Institute

John H. BORDEN*, Ph.D. (California) Professor of Biological Sciences, Simon Fraser University (Burnaby, B.C.)

Lawrence FOWKE*, Ph.D. (Carleton) Professor of Biology, University of Saskatchewan (Saskatoon, Sask.)

Bryan L. HARVEY*, Ph.D. (California) Professor and Head of Crop Science and Plant Ecology, University of Saskatchewan; and Director of the Saskatchewan Crop Development Center (Saskatoon, Sask.)

Wilfrid A. KELLER*, Ph.D. (Saskatchewan) Head, Genetic Engineering Section, Plant Research Centre, Agriculture Canada (Ottawa, Ont.)

Ann OAKS*, Ph.D. (Saskatchewan) Professor of Biology, McMaster University (Hamilton, Ont.)

A. C. OEHLSCHLAGER*, Ph.D. (Oklahoma State) Professor of Chemistry, Simon Fraser University (Burnaby, B.C.)

Trevor L. THORPE*, Ph.D. (California) Professor of Biology, University of Calgary (Calgary, Alberta)

G. H. Neil TOWERS*, Ph.D., (Cornell) Professor of Biology, University of British Columbia (Vancouver, B.C.)

Guest Researchers at the Institute*

- Dr. Garth Abrams, University of Saskatchewan
Mr. Sola Akingbala, University of Ibadan
Dr. Jesus Alvaraz, Government of Spain
Ms. Alison Baillie, University of Saskatchewan
Dr. V.K. Bansal, Agriculture Canada
Ms. Colleen Bennett, Manpower Temporary Services
Ms. Lesley A. Boyd, University of Saskatchewan
Mr. Robin Browne, University of Saskatchewan
Mr. H. Cheng, Genetic Institute of Jiangsu Academy of Agricultural Sciences, Nanjing, China
Mr. W. Cramerstetter, Cramer Nursery Inc.
Dr. Normand Chevrier, Université du Quebec
Mr. Jim Cunningham, Philom Bios Inc.
Dr. Hélène David, Université de Bordeaux
Dr. Laurence Davin, Université Paul Sabatier
Mr. A. Dolemeyer, University of Saskatchewan
Dr. Udo Ellert, University of Braunschweig
Ms. Jackie Frovarp, University of Saskatchewan
Dr. Basu V. Gantotti, Philom Bios Inc.
Dr. Phil Gorin, Univ. Federal do Parana, Brazil
Ms. Linda Gray, University of Saskatchewan
Ms. Catherine Greuel, Philom Bios Inc.
Ms. Sallaja Gummurulu, University of Saskatchewan
Mr. D. Marcus, McGill University
Dr. George Haughn, University of Saskatchewan
Ms. Harriet Hemmingsen, Queen's University
Mr. Gordon Holtslander, Philom Bios Inc.
Mr. Barry Holwerda, University of Saskatchewan
Mr. L. Hongchao, Beijing Academy of Agricultural Science
Ms. Doreen Hnatko, Industrial Research & Development Ltd.
Mr. B. Jarvis, University of Saskatchewan
Ms. Funmi Joseph, University of Saskatchewan
Ms. Susan Kikcio, University of Calgary
Ms. Kristina Komendant, Philom Bios Inc.
Ms. Cathy Kulack, Philom Bios, Inc.
Ms. Laurie Litwinson, University of Saskatchewan
Mr. Ben Lui, Philom Bios Inc.
Dr. C. Mak, University of Saskatchewan
Ms. Arlene Malone, Philom Bios Inc.
Mr. Jim Malone, Philom Bios Inc.
Ms. Jocelyne Martel, Manpower Temporary Services
Ms. Linda McGregor, Saskatchewan Wheat Pool
Mr. E. Nater, University of Saskatchewan
Mr. N. Nehra, University of Saskatchewan
Mr. Y. Niu, University of Saskatchewan
Dr. Brian Orshinsky, Saskatchewan Wheat Pool
Dr. E. Perucco, Italian National Research Council
Ms. Ruth Powers, University of Saskatchewan
Mr. Martin Reaney, University of Saskatchewan
Ms. Charlotte Rijnders, Leiden University, Netherlands
Mr. Kevin Rozwadowski, University of Saskatchewan
Dr. P.K. Saxena, University of Saskatchewan
Mr. Brent Selinger, University of Saskatchewan
Dr. N.P. Shayo, University of Saskatchewan
Dr. John Sheppard, Biotechnology Research Institute
Ms. Sharon Sprout, University of Saskatchewan
Mr. Dan Stewart, Blueberry Acres Ltd.
Mr. Tom Tautorius, Stange Canada Inc.
Dr. Leena Toivonen, Finnish Academy of Science
Mr. Hong Wang, University of Saskatchewan
Ms. Linda Wong, University of Saskatchewan
Ms. Adrienne Woytowich, University of Saskatchewan
Mr. Zinjie Xia, University of Saskatchewan
Mr. Jia Xia, University of Saskatchewan
Ms. P. Yeung, Women in Technology

3. Cell Technology Laboratory

Laboratory Head: Dr. K.K. Kartha

3.1 Overview

The Cell Technology Laboratory develops and uses in vitro techniques of plant cell culture. These techniques have been a strength of the Institute ever since it pioneered plant cell culture in the 1960s; the Laboratory continues to provide some of Canada's best know-how and scientific facilities in this field. Current projects on Crop Biotechnology and Conifer Biotechnology develop and use cell manipulation methods in two traditionally difficult systems: monocot plants such as wheat, barley

and corn; and gymnosperms such as conifer trees.

During 1987/88 the Laboratory expanded through construction of a new conifer laboratory and by acquisition and enlargement of another Laboratory unit for molecular biology work. Added growth rooms and cabinets improved the Laboratory's facilities for cell and plant experimentation while through a series of equipment purchases we strengthened our molecular biology capacity.



Wheat inflorescences tagged to mark date of anthesis in order to collect embryos of desired age.

3.2 Crop Biotechnology Project

3.2.1 Project Personnel

Research Officers

K.K. Kartha (leader), F. Constabel, A. Cutler,
P. Dawson, F. Georges, K.N. Kao, R. Reichert,
M. Saleem

Research Associates

J. Boothe, R. Chibbar, E. Kendall, J. Newsted,
J. Qureshi

Technical Officers

K. Caswell, M.A. Coffey, D. Horn, N.L. Leung,
M.K. Loewen, C.S. Mallard, M. Michayluk, K. Pahl,
B. Papish, S. Polvi, J.P. Shyluk, L. Steinhauer

Guest Researchers

S. Akingbala, A. Baillie, H. Cheng, W. Cramerstetter,
N. Chevrier, A. Dolemeier, L. Hongchao, C. Mak,
L. McGregor, E. Nater, N. Nehra, Y. Niu,
B. Orshinsky, P.K. Saxena, B. Selinger, N.P. Shayo,
J. Sheppard, H. Wang, L. Wong, J. Xia

Summer Assistants

S. Girard, T. Legros, H. Vanzegeren

3.2.2 Objectives

(1) To produce prototype wheat and barley plants with specific added qualities of resistance to herbicides and tolerance to cold;

(2) To produce prototype oilseed plants with frost or herbicide resistance.

3.2.3 Progress in 1987/88

This year, project researchers organized their work around two foci: *in vitro* techniques for cell selection and regeneration of wheat and barley; and the molecular biology of cell tolerance to cold and of cell regeneration.

3.2.3.1 Cereal Cell Technology

Work began in 1986/87 on *in vitro* manipulation of cereals. The objective includes selection of herbicide resistant and frost tolerant varieties in spring wheat¹. A major and limiting problem in cereal biotechnology is the difficulty of regenerating whole plants reliably from cultured cells, especially from protoplasts. Researchers directed a substantial portion of their year's work at this problem which is most severe with wheat.

Wheat regeneration systems

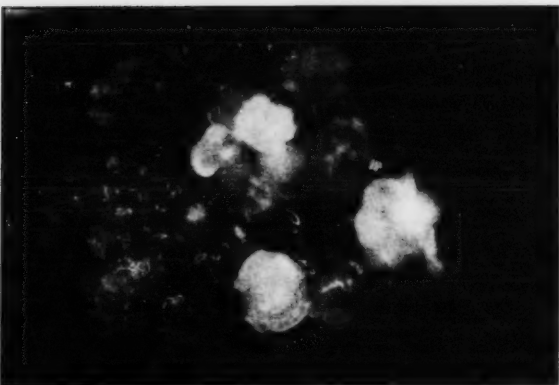
We developed procedures to culture tissues of various spring wheat genotypes *in vitro*. The genotypes studied varied in their ability to produce morphogenic or embryogenic callus, giving both superior and inferior genotypes (in terms of *in vitro* culturability). For future variety development programs, incorporation of traits responsible for *in vitro* culturability may itself be desirable to make varieties available for genetic manipulations. From the germplasm available, we selected one genotype highly responsive in culture, and another showing a very poor response. By performing reciprocal crosses between the two, we recovered hybrids showing high *in vitro* regeneration capability. By

¹ A portion of the work on wheat was carried out as a joint research venture with the Saskatchewan Wheat Pool assisted by NRC's Industrial Research Assistance program.

Figure 1



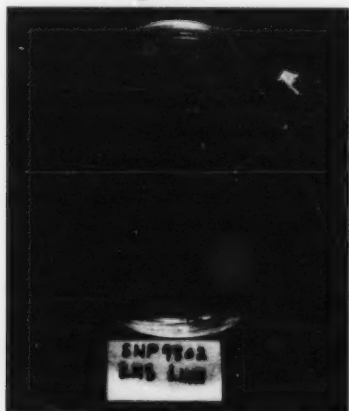
Culture of immature embryos collected 21 days post anthesis. Notice germination in the absence of abscisic acid (ABA), and complete suppression of germination in the presence of ABA.



Callus obtained from older embryo in the presence of ABA, note the abundance of somatic embryoids (above) and somewhat developed somatic embryos (left).

developing a closely monitored callus line subculture routine, we have now overcome the problem of diminishing regeneration potential of cereal callus over time. This problem has plagued efforts toward in vitro selection for a long time. Now we can initiate morphogenic or embryogenic cultures, and maintain them indefinitely in that state. This permits really meaningful experiments.

Figure 2



A callus line after two year's subculture. No loss of regeneration capacity.

New explant material for wheat cultures

Cereals offer a very limited choice of explant material for morphogenic or embryogenic callus. The most common and reliable source of such cultures is the immature embryo. In our experience, immature embryos collected 10 to 14 days after anthesis, possess scutella which proliferate upon culture to give callus. Embryos collected more than 14 days after anthesis showed a very poor response in culture; they generally possessed quiescent scutella and readily germinated. Very little or no callus is produced from these embryos, and furthermore, the callus is usually incompetent for plant regeneration. Addition of abscisic acid to mature excised embryos renewed expression of several embryogenic sequences. We have now shown that abscisic acid supplements restore juvenility in older

embryos. Through this method old embryos' regeneration increased as much as tenfold. These findings permitted competent cultures from those embryos which earlier seemed useless, and so have increased the range of exploitable explant material.

Somaclonal variation in wheat

Variation induced at the micro level in vitro, gives non-uniform cultures which can be useful sources of whole-plant variation. We generated a large population of variant plants (somaclones) from several wheat genotypes (cultivar HY320) and took regenerated plants for field evaluation during 1987. There was variation in plant height, maturity, kernels per spike and hundred kernel weight. We identified several prospective variants for 1988 yield trials.

In vitro selection for herbicide resistance

Our ability to bulk and maintain competent callus cultures of wheat has enabled us to perform in vitro selection experiments against glyphosate. Glyphosate is a potent post-emergence herbicide against a variety of weeds, but the susceptibility of wheat cultivars to this herbicide limits its use.

We searched for and found resistance at the cell level. We allowed cells which survived exposure to high glyphosate levels to regenerate whole plants then took these plants to maturity and collected seed for planting and evaluation in 1988.

Characterization of cryoselected wheat callus

The ability of a plant to withstand severe freezing stress seems related to the composition of its membranes. For example, during cold acclimation membrane lipids undergo progressive desaturation, and α -tocopherol levels change. In addition, changes in membrane proteins component have been reported. The relation of membrane changes to freezing tolerance is the topic of recently-begun

collaborative research by PBI² whose research aims include: understanding survival mechanisms in cryoselected callus lines; elucidating the role of antifreeze protein in depressing the freezing point of plant tissue; defining a role for α -tocopherol in stabilizing membranes.

Biochemical analysis of callus line SWP9302 showed no differences in total fatty acid levels among parent (9302), mutagenized (9302EMS) and mutagenized cryoselected (Star) lines, although 9302EMS and Star contained relatively more sterols and saturated fatty acids than did the parent line.

Determinations of the efficiency of fish antifreeze protein in depressing the freezing point of plant tissues used differential thermal analysis to measure freezing activity in leaves of *Arabidopsis thaliana* infiltrated with antifreeze protein. We observed significant freezing point depression at initial protein concentrations of 0.1%. The effect was specific for antifreeze-type proteins. This demonstration of efficacy has opened the way for genetic engineering attempts in 1988.

Barley regeneration systems³

PBI researchers developed systems for high frequency plant regeneration from immature embryo cultures of Ellice and Heartland barley. We evaluated embryos plated on medium containing various levels of the auxins 2,4-D and 2,4,5-T for plant regeneration. Precocious germination of the embryos decreased as the auxin concentration in the medium increased. Up to eighty-five percent of all Ellice embryos produced shoots (mean number

per callus was 4.3). Fifty-five percent of Heartland embryos produced a mean of 3.7 shoots per callus. Argyle and Harrington embryos produced very few or no shoots on any of the media tested.

We transferred the shoots produced to root formation, then after medium several weeks put the plantlets into soil in the greenhouse. Several hundred plants have been grown to maturity and the heads collected for 1988 field trials to assess uniformity.



G-bands in a metaphase cell.

Barley anther culture⁴

A great amount of time can be saved by using haploid methods for plant breeding. The objective of this work is to improve haploid techniques for this purpose.

We achieved better induction of green plants from microspores by anther culture methods in two-rowed barley in 1987, with up to 13 green micro-

² Collaborators: Prof. B.D. McKersie (Guelph University) and Prof. L.V. Gusta (University of Saskatchewan).

³ We carried out some aspects of this work in collaboration with the Department of Crop Science and Plant Ecology (University of Saskatchewan).

⁴ We carried out some aspects of this work in collaboration with the Department of Crop Science and Plant Ecology (University of Saskatchewan).

spore-derived plants obtained per anther. We can now obtain green microspore-derived plants from any of the lines or varieties tested (including Bonanza) which only produced albinos a few years ago. Further effort is under way on the reduction of the frequency of albinos.

Over one thousand green microspore-derived plants were produced during 1986 and 1987. About 70% of them were spontaneously doubled. The Crop Development Center evaluated their seeds in the field under a testing contract. Results from 1987 trials indicated that average performance of lines developed by different methods was the same. However, the anther culture method permitted net-blotch resistance to be rapidly transferred from six-rowed barley to two-rowed barley.

G-banding in plant chromosomes

The G-banding technique is very useful for identifying individual chromosomes in somatic hybrids. The technique is well developed in mammalian chromosomes but has failed for plant chromosomes despite attempts by many cytogeneticists in the past few years. During 1987 we successfully developed a technique to produce G-banding in plant chromosomes⁵.

3.2.3.2 Molecular Biology

Cereal protoplast recalcitrance

The ability to regenerate plants from protoplasts is fundamental to the success of many cell selection and genetic engineering operations. With some plant species such as tobacco, regeneration is now

easy, but with others - including the cereal grains - it is still scarcely possible. The objective of this work is to overcome the "recalcitrance" of these latter species.

A major cause of cereal leaf protoplast instability is oxidative stress. We alleviated this and thereby stabilized the protoplasts by adding the anti-oxidant n-propyl gallate. Strikingly lower peroxidase activities distinguish cereal leaf protoplasts from cereal suspension culture protoplasts capable of mitosis and normal development. Excessive enlargement and budding, the normal fate of cereal leaf protoplasts, imply aberrant cell wall deposition. Various anionic peroxidase forms that appear rapidly in suspension culture protoplasts appear to mediate cell wall formation and mitosis. We have found these to be synthesized *de novo* and have shown that some are specifically associated with meristematic and totipotent leaf base areas in corn and wheat. Further evidence for the role of peroxidases in proper cell wall formation came from bromegrass suspension cultured protoplasts treated with peroxidase inhibitors, which under these conditions showed increased budding and enlargement in otherwise normally dividing protoplasts.

Freezing tolerance in plants

A 1986/87 Plant Biotechnology Fund contract⁶ provided quantities of pure antifreeze protein (AFP) from the flounder fish for experiments on the use of the protein in frost protection in plant tissues. With *Arabidopsis thaliana* leaf cells, in the presence of an excess of ice to act as a nucleator, infiltration of AFP produced no significant benefit; tissue freezing points were not significantly affected.

⁵ Collaboration: Prof. H.C. Wang (Anatomy, University of Saskatchewan).

⁶ Contract (1986/87): Memorial University of Newfoundland: Prof. G. Fletcher.

However, in the absence of ice nucleation AFP allowed several degrees of tissue supercooling. Therefore, this protein seems useful in protecting susceptible plants (such as potato or spring canola) against untimely frosts. Having demonstrated an effective anti-freeze protein in plant cells, we continued efforts to engineer plants with its gene. We engineered a plant vector harbouring a synthetic gene corresponding to the flounder AFP as described previously, for electroporation into plant protoplasts. First experiments have shown measurable expression.

Markers of embryogenesis

We completed the search for biochemical/ molecular markers of embryogenesis with cell cultures to be used for the selection of material competent for regeneration. In extracts of carrot cell suspensions aryl esterase isozymes were identified as indicative of somatic embryogenesis. The electrophoretic pattern of this esterase was the same in cell suspensions competent for embryogenesis as in suspensions of embryos at various stages of development.

3.3 Conifer Biotechnology Project

3.3.1 Project Personnel

Research Officers

D. Dunstan (leader), A. David (visiting),
M. Lapp, E.W.T. Tsang

Technical Officers

T. Bethune, C. Bock, J. Malinek

NSERC Fellow

F. Bekkaoui

Guest Researchers

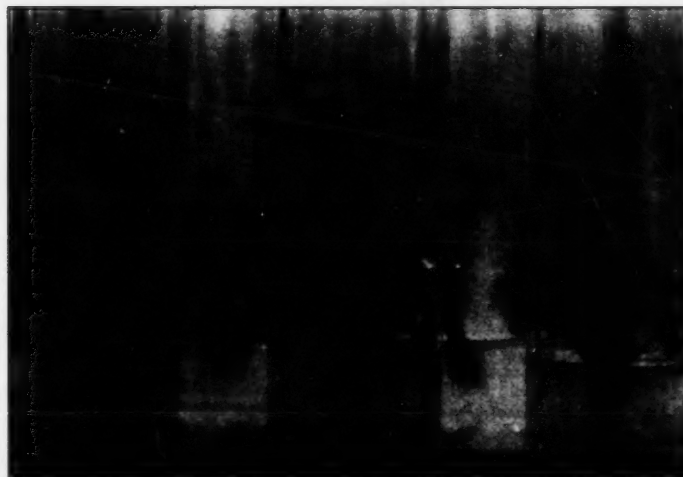
R. Browne, H. David, D. Hnatko, S. Kikcio,
D. Stewart, T. Tautorus

Summer Assistants

D. Glaeske, M. Pilon

3.3.2 Objectives and Background

Canada's forests of the future must contain trees capable of quality wood production for lumber, pulp and paper applications. Fast growth, resistance to disease, and tolerance to environmental stresses such as insect pests, climate, and soils. The objective of this project is to develop enabling biotechnology for the propagation and improvement of coniferous forest trees for the provision of superior, elite, or genetically engineered trees. The technologies being developed at PBI to achieve such trees are Plant Propagation (organogenesis, somatic embryogenesis) and Plant Improvement (protoplast culture and genetic transformation).



Douglas Fir shoots produced from dormant winter buds as a micro-propagation technique.

3.3.3. Progress in 1987/88

3.3.3.1 Plant Propagation

Working with Douglas fir, we have optimized lateral bud induction and primary shoot elongation. We worked on optimizing remultiplication, and achieved a slow root induction on shoots which had been remultiplied over the past two years. We failed however, in improving survival of the initial bud explants during the critical period between 9 and 15 weeks after inoculation.

We have developed a technique for high level lateral bud induction on explants from *Picea glauca* but we have not yet been able to promote consistent and satisfactory primary shoot elongation.

Douglas fir (*Pseudotsuga menziesii*)

Research in 1987/88 concentrated on reduction in explant losses between 9 and 15 weeks after inoculation. Our previous studies had indicated that 0.4 g/L ammonium nitrate in media was optimal for lateral bud induction, when included only for the first six weeks of culture⁷. Work continued on optimising sucrose concentration and showed that 2.5% was the level of choice. Photoperiods of 24 h and 16 h gave indistinguishable results, as did fluorescent and incandescent lighting. Benzyladenine induced lateral buds in remultiplied shoots. These were grossly abnormal following 3 weeks of treatment but more or less normal following 1 week of treatment, suggesting that pulse applications of the chemical might be more effective. We also tried promotion of shoot quality and quantity in a remultiplying system, and induction of roots on remultiplied shoots *in vitro*. Rooting experiments examined effects of auxins, of salts and of sucrose. Callus

induction was common to all treatments though the amounts of callus on control shoots was least. To date 6 shoots have been recovered (1% of total) which have distinct root-like structures. These structures have not elongated in agar medium nor in Knop's solution. No one particular treatment favoured their production. Extensive organ culture attempts with white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*) did not produce satisfactory cultures.

Somatic embryogenesis studies in 1987/88 used abscisic acid analogs to stimulate the development of somatic embryos of conifers into transplantable plantlets. In suspension culture somatic embryos remained in a repressed state of development and could multiply themselves approximately three-fold each week. We achieved promotion of embryo maturation into plantlets, using abscisic acid itself, incorporated into an agar-solidified medium for a period of 35 days. However, we recovered only 2% of the potential embryos originally plated. Induction of somatic embryogenesis from zygotic embryos of Jack pine (*Pinus banksiana*) has proved even more difficult.

Western white pine (*Pinus monticola*)⁸

We improved shoot production from embryos through modification of media and by using benzyladenine stimulation. There were significant interactions between some of the parameters tested. Shoot elongation and rooting rate was increased to a "best yet" figure of 50%. Rooted shoots have been moved to a soilless forestry mix with 413 of 509 plantlets surviving to date. This has been the project's most successful species in terms of rooting ability during micropropagation.

⁷ Collaboration: Profs. T. Thorpe and M. Maloney (Biology, University of Calgary), and K. Patel (AgriForest Technologies Ltd., Kelowna).

⁸ Collaboration: Canadian Forestry Service (Victoria).

3.3.3.2 Plant Improvement

Protoplasts⁹ and genetic engineering

This research clarified the requirements for the isolation of protoplasts and subsequent regeneration of somatic embryos, from embryogenic white spruce suspensions. It has not yet been possible to recover plantlets from this regeneration material, however the achievement of embryos from protoplasts was a major breakthrough which allowed gene transfer work to be set in motion. Direct gene transfer and co-cultivation experiments were begun at the end of 1987 to establish protocols and to examine different possible means of transfer of marker genes. White spruce has been the main target species. By March 1988 putative transgenic cells were in hand.



Agrobacterium attachment to protoplast-derived white spruce cell (lower left).

⁹ Collaboration: Prof. L. Fowke (Biology, University of Saskatchewan).

4. Gene Technology Laboratory

Laboratory Head: Dr. W.L. Crosby

4.1 Overview

The Gene Technology Laboratory is concerned mostly with DNA recombinant techniques for genetic engineering of plants. Established as a major thrust of the Institute only in 1984, gene technology is providing powerful tools for crop improvement not possible through other approaches. The Laboratory aims to provide a high-quality physical and intellectual facility accessible by Canadian research and development interests. Plant Molecular Genetics and Plant Productivity Genes, the two

projects based in this Laboratory, have already forged strong links with other PBI activities as well as with external collaborators.

The Laboratory added two working units during 1987-'88, for a current total of seven, and acquired several key pieces of equipment including an oligonucleotide synthesizer to service external as well as internal needs.



Technical Officer Maureen Dennis measures peak heights on immune electrophoresis rocket gel.

4.2 Plant Molecular Genetics Project

4.2.1 Project Personnel

Research Officers

W. L. Crosby (leader), S. M. Hemmingsen,
L. E. Pelcher, G. Selvaraj, J.L. Taylor

Technical Officers

C. Barber, C. Bennett, J. Condie, J. Hammerlindl,
R. Hirji, D. Kneeshaw, M. Olson, D. Schwab,
M. Wood

Research Associates

A. Hussain, A. Good, D. Raju

NSERC Fellow

L. Klimczak

Guest Researchers

C. Bennett, L.A. Boyd, D. Harcus, H. Hemmingsen,
B. Holwerda, E. Perucco, K. Rozwadowski,
A. Woytowich, Z. Xia

Summer Assistants

M. Lavesque, A. Woytowich

Dr. Sean Hemmingsen (left) of the Gene Technology Laboratory talks with Dr. Warren Steck, Institute Director.

4.2.2 Objectives and Background

The objectives of this project are to create and develop enabling gene technology for the genetic transformation of higher plants. This free-ranging objective reflects the relative newness of this line of work at NRC, its fundamental nature, and the need for project flexibility in a research field which is advancing at an explosive rate.

The year brought high achievement, considerable project strengthening, new staff and a recognition of excellence in the form of two President's Awards. New Research Officers, Sean Hemmingsen and Janet Taylor, set up and activated two new project laboratories which are now in full operation. During 1987/88, we began funded collaborations with other government laboratories and with Biotechnica Canada Inc., in addition to the core work of the project.

We continue to emphasize sophisticated recombinant DNA techniques for the characterization, insertion and expression of non-plant genes in plants. Tobacco and Arabidopsis thaliana have proven useful experimental species, with canola (Brassica napus) an ultimate focus of attention.



4.2.3 Progress in 1987/88

4.2.3.1 Genetic Engineering Techniques for Canola

The aims of this project are to create and use reliable techniques for transfer of some foreign genes into *B. napus*. Genes encoding tolerance to sulfonylurea herbicides have been functionally expressed in tobacco and *B. napus* and a new tolerance gene has been isolated and sequenced from canola. Through genetic engineering, we have produced herbicide resistant plants in our laboratories.

Improvements in our transformation protocols allowed several foreign genes to be successfully introduced into canola, and shown to be stably inherited. An interesting example of this has been our transfer of modified genes encoding for insecticidal *Bacillus thuringiensis* toxins into tobacco and canola.

The group continued development of better vectors centered on mini TI plasmids and specialized *Agrobacterium* systems.

Research progress during the past year can be summarized as follows:

1. We constructed several mini TI plasmid vectors and tested them for their ability to transform tobacco. These vectors were designed for maximal stability in appropriate *Agrobacterium* host strains, which were engineered to provide all required trans-acting replication functions. The vectors included several useful features:

- multiple cloning sites within a lac-alpha complementation region;
- ability to replicate as single-stranded DNA;
- packageability in M13 and bacteriophage lambda. The small vector size (about 12 kilobases),

large copy number in *E. coli*, and stability in *Agrobacterium* host strains offer distinct advantages. Further modifications of these vectors to afford other uses are in progress.

2. Researchers have isolated and sequenced a family of Hind III repetitive DNA elements from *Brassica napus* genome. We found several mustard family species that harbour this element. We identified a potential progenitor of the element in *B. campestris* and *B. oleracea*. An unusual organization of this sequence in these plants spurred our interest in studying the origin and evolution of this family of repeated DNA.

3. Preliminary experiments on functional equivalence of individual T-DNA borders in promoting gene transfer to plant cells was the object of a President's Fund award being carried out with Drs. Selvaraj, Crosby and Raju. They constructed a set of vectors with variable left and right borders, while our sequencing unit confirmed the placements of these borders by DNA sequence analysis. Additional constructs were required before carrying out plant transformation experiments.

While searching for anti-stress genes which will be important in genetic engineering of Canadian crops, we isolated and characterized a series of bacterial genes encoding for salt-tolerance, and genes involved in plant responses to pathogens. The former seem similar to genes in salt-tolerant plants. Extensive transposon insertion analysis revealed three functional domains necessary for full levels of salt tolerance.

4.2.3.2 Genes Regulating Plant Cell Proliferation

The processes by which plant cells grow, divide, multiply and specialize in an orderly way to produce plants are central to plant biotechnology. Regulation of cell multiplication is an important goal of this sub-project. Its objectives during its opening two

years are to use appropriate full-length cDNA libraries from plant-derived mRNA to examine complementing sequences for specific mutations (in yeast). Over-expressions in plants may allow cell cycle phenotypes to be assessed.

We grew appropriate plant material and extracted nucleic acids tissue. The poly A+ fraction of the RNA has been purified for use in cDNA synthesis.

We obtained three wild-type Saccharomyces pombe genes which give dosage-dependent cell cycle phenotypes, and subcloned each of these into vectors suitable for the application of site-directed mutagenesis. One of the yeast sequences has been engineered for insertion into a Ti plasmid for introduction of constructs into tobacco and Arabidopsis thaliana.

4.2.3.3. Oligonucleotide Synthesis and Sequencing

The Plant Biotechnology Institute's original (1984) automated oligonucleotide synthesizer provides DNA strands for in-house use as well as for collaborators and clients. In 1987/88 the Institute decided to set up an additional cost-recovery synthesis service and, in view of increasing demand, to dedicate a second gene machine entirely to this purpose. In March 1988 we received a BioSearch 4000 synthesizer following several months of testing and comparison of instruments. A technical officer was trained in the use of the machine, and the service has turned out its first oligonucleotides satisfactorily. We are now integrating synthesis and sequencing operations into a single unit.

We set up a first-class sequencing facility during 1987 and by March 1988 were sequencing 3000 bases monthly with outputs of unusually high quality. We completed sequences of cDNA for all three members of the barley alcohol dehydrogenase gene family, and are sequencing -- as yet incomplete -- more than seven other DNA strands at year's-end.



Technical Officer Rozina Hirji displays bacterial isolates used to transfer DNA into cells.

4.3 Plant Productivity Genes Project

4.3.1 Project Personnel

Research Officers

J.D. Mahon (leader), S.L.A. Hobbs, L. M. Nelson

Technical Officers

D. Baliski, C. DeLong, M. Dennis, C. Knapp

Research Associates

C. Chanway, M-F. LeGal

Guest Researchers

F. Joseph, G. Sallaja, S. Sprout, M. Wilson

Summer Assistant

M. Wilson

4.3.2 Objectives and Background

Relative to viral, bacterial and yeast genes, the eukaryotic genes of green plants have been little studied and are not so well known. Yet plant-derived genes are needed for genetic engineering plant improvement in years ahead. The objective of the project is to identify and characterize genes controlling agriculturally important traits of crop plants and associated microorganisms.

4.3.3 Research Progress in 1987/88

4.3.3.1 Soil Bacteria and Crop Performance

Some soil microorganisms promote the growth of crop plants, although the mechanism remains unknown. Recent field tests by both private and public developers of microbial inoculants have

encountered very high variability in field responses. At PBI, we are assessing plant growth promotion by soil bacteria with two systems: legume/Pseudomonas and wheat/Rhizobium.

In collaboration with Allelix Inc., some of the company's Pseudomonas strains have been tested with pea and lentil. No growth stimulation was seen with peas, but one strain consistently stimulated root growth of the lentil variety Laird by 20 to 30%. The variety Eston, however, did not respond to inoculation with this strain, showing that there are very specific plant-bacterial interactions. No stimulation of symbiotic activity was noted with any of these strains, even those which had been selected to promote nitrogen fixation in soybeans. This high genetic specificity suggested that bacteria promoting the growth of specific cultivars might be found in the presence of these cultivars. To examine this hypothesis, soil samples were collected from a field in which wheat had been grown for 27 years (with the variety Katepwa for the last 5). Bacillus isolates obtained from these soil samples, when tested for their growth promoting activities in Katepwa, Neepawa and HY320 varieties of wheat, produced root dry weight increases (up to 38%) in Katepwa only. Katepwa and Neepawa being very closely related, suggests that the isolation of "plant-adapted" strains may be a simple but effective way to secure agronomically beneficial microbes.

Field trials in collaboration with Esso Chemicals Ltd. tested 14 strains of Rhizobium leguminosarum, previously shown effective in controlled environment studies, as lentil inoculants. Results were clearly superior to commercial inoculants currently in use.

In collaboration with the University of Saskatchewan, we determined the effects of the herbicide metribuzin on lentils inoculated with

Rhizobium leguminosarum strains. Some inoculant strains were completely herbicide resistant and one increased plant growth. Because symbiotic activity was dependent on strain but not metribuzin application, it may even prove possible to select strains with both symbiotic effectiveness and herbicide tolerance.

4.3.3.2 Genetic Control of Photosynthesis

The enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) strongly determines primary plant productivity. As a bifunctional enzyme it controls the rates of both carboxylation of ribulose-1,5-bisphosphate (leading to sugar formation) and oxygenation of the same substrate leading to the apparently wasteful photorespiratory cycle.

Recently we demonstrated that the photosynthetic capacity of different pea genotypes is strongly related to the activity of this enzyme in the leaves. Because the production of this enzyme in response to light is controlled by the transcription of mRNA from the small subunit genes, genetic variability in RUBISCO could be examined by exposing dark-grown plants of various genotypes to light and following the appearance of RUBISCO in the tissues. From the same tissues, researchers isolated RNA and hybridized it to a probe produced from a pea small sub-unit cDNA clone. The results revealed strong correlation between leaf RUBISCO and sub-unit mRNA levels. Moreover, this correlation held in both field and controlled environment experiments. If this indicates genotypic control of the mRNA transcription rate, it may be possible to identify the sequence differences controlling this variability.

Photosynthesis is also affected by the level of carbon dioxide to which leaf tissues are exposed, and CO₂ enhancement is a common technique for increasing productivity in commercial glasshouse crop production. But the carboxylase and oxygenase functions of the enzyme are competitive, so

adding CO₂ simultaneously increases photosynthetic CO₂ fixation and decreases photorespiratory carbon loss. When we switched peas grown at normal CO₂ levels to high CO₂, photosynthesis and shoot growth both increased as expected. However, the amount of RUBISCO protein decreased, as did the carboxylase activity, and the amount of small sub-unit mRNA decreased relative to the total mRNA. Because the latter did not change, high levels of CO₂ must restrict the transcription of RUBISCO small sub-unit mRNA.

Carbon dioxide fertilization is not possible for field crops, but even here photosynthesis to photorespiration ratio could be increased by modifying the relative affinities of the enzyme for the two substrates. Recently it has been shown that the affinity of RUBISCO for CO₂ varies among photosynthetic organisms. If the protein differences controlling this variation could be identified, directed modification of RUBISCO genes would be feasible. Because the large subunit has the binding sites for all of the substrates, it has been extensively studied. There are at least 60 amino acid residues which differ between the low-affinity species group and the high-affinity group from which the genes have been sequenced. In collaboration with Dr. Paul Whitfeld of the Australian CSIRO, we examined these rbcl genes from two closely related species of Flaveria. DNA fragments 2300 bp long, containing the complete coding regions were isolated from each species, cloned and sequenced in both directions. The gene sequences differed in only 8 nucleotides and the deduced amino acid sequences in three residues. This result has greatly narrowed the range of possible amino acid changes which might be involved in determining the enzyme's affinity for CO₂.

5. Biological Chemistry Laboratory

Laboratory Head: Dr. E.W. Underhill

5.1 Overview

PBI's Biological Chemistry Laboratory puts a strong "molecular" element into molecular biology at the Institute. Through its resources for structure/function analysis, by synthesis of biomolecules, and in plant biochemistry, the Laboratory provides a first-class facility for advanced research in these fields. The Institute has a long tradition of excellence in phytochemistry and in plant biochemistry, now manifested by two projects: the Bio-organic Chemistry project includes excellent organic chemical analysis facilities, while the Plant Cell

Metabolism project links biochemistry with cell biology to produce unique new industrial technologies.

The year brought improved facilities for the Laboratory. A centre for accommodation of 10 more guest researchers enabled important new collaborations to proceed, while a new and sophisticated large-molecule mass spectrometer advanced the analytical abilities of Biological Chemistry both for NRC projects and for external users.

Dr. Suzanne Abrams examines a Dreiding stereo model.



5.2 Bio-organic Chemistry Project

5.2.1. Project Personnel

Research Officers

E.W. Underhill (project leader), S. R. Abrams,
J. W.D. GrootWassink, S. L. MacKenzie,
M. Mazurek, G. P. Slater

Technical Officers

D.L. Barton, M. Giblin, L.R. Hogge, L.L. Hoffman,
A.D. Kolenovsky, D.J. Olson, D.W. Reed,
A.C. Shaw, D.M. Tenaschuk

Research Associates

J.C. Jain, R. Martel, K.A. Pivnick, D.C. Taylor,
L.W. Thomson, R.J. Weselake

Guest Researchers

G.D. Abrams, V. Bansal, L. Davin, J. Frovarp,
G. Haughn, B.J. Jarvis, C. Kujack, L.A. Nelson,
M.S. Pedras

Summer Assistants

W. Stirton, L. Litwinson

5.2.2 Objectives and Background

A significant change in research direction took place during 1987 with increased focus on plant biochemistry. The start-up delays associated with this change are now behind us and we are making good progress in the new research and in developing extramural contacts and collaborations. The project's objectives for the past year included: (1) synthesis and field applications of abscisic acid analogs, (2) applications of plant and insect derived semiochemicals for protecting crops from insects,

(3) isolation and characterization of plant lipases: their physiological functions and industrial applications, and (4) modification of phytochemical phenotypes through DNA technologies.

The project continued to operate PBI's molecular analysis laboratories.

5.2.3 Research Progress in 1987/88

5.2.3.1 Bio-organic molecules

Abscisic acid-type regulators

Abscisic acid is one of the most important plant hormones, with roles in all stages of plant development: seed germination, growth, transpiration and adaptation to environmental stresses such as drought and cold. Moreover, this natural compound occurs universally in plants.

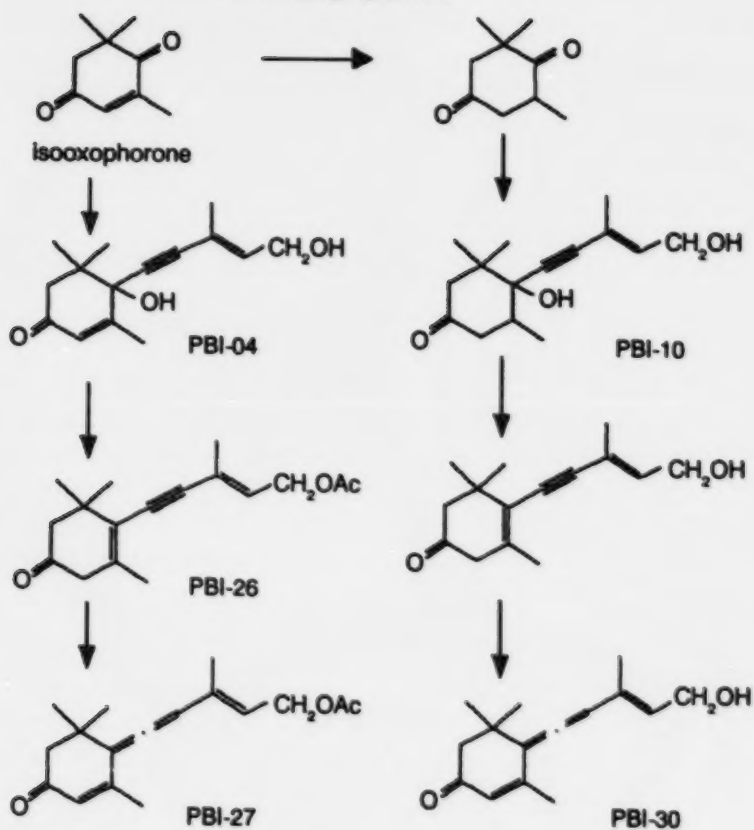
Yet abscisic acid is not useful in agricultural field applications. It is expensive, poorly absorbed by plants and - most important - rapidly degraded biologically. This project's main aim is to synthesize molecular analogs of abscisic acid which will prove useful as plant regulators. This involves, on one hand, structure-function tests and on the other, evaluation of breakdown metabolism of abscisic acid and promising analogs. Numerous collaborators are involved in testing of biological activities, and PBI awarded a testing contract under the Plant Biotechnology Fund's auspices.

We have synthesized and examined more than two dozen analogs. Several are strong regulators of plant development in our tests, and some perform so well that crown patent action has been initiated. We will field-test selected compounds from 1987 tests more extensively during the summer of 1988.

Host-specific toxins of Blackleg fungus

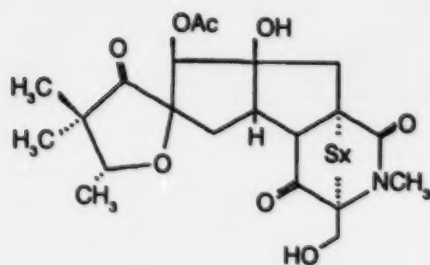
The fungus *Phoma lingam* causes blackleg disease in rapeseed, resulting in severe yield losses. Cana-

Figure 3
ABA Analog Synthesis



trans series shown; cis series proceeds similarly

Figure 4



x = 1 sirodesmin H
x = 3 sirodesmin J
x = 4 sirodesmin K

dian spring canola varieties are not resistant and present treatment methods are not effective. This project, a collaboration with Agriculture Canada, aims to isolate and identify toxins of *P. lingam* which might be host-specific, for use in *in vitro* selection searches for resistant rapeseed cells.

Our 1986/87 work isolated two main toxins (sirodesmin PL and deacetylsirodesmin PL) which however proved to be non-specific in biological assays. We have also isolated three minor but structurally related toxins (sirodesmin H, J and K). These novel substances, whose structures were confirmed by synthesis, closely resemble sirodesmin PL in toxicity and non-specificity. Soon final experiments will bring this project to a close.

Plant substrates affecting insect behaviour

Many volatile plant chemicals assist insect pests to locate suitable hosts. Sometimes related plants are differently susceptible to pests. This project's objective is to identify chemical attractants or deterrents demonstrated in a few target systems.

The diamondback moth prefers brown mustard (*Brassica juncea*) for oviposition. Chemical extractions implicated hydrolysis products of the glucosinolates present abundantly in this species. We found ovipositing moths equally attracted by allyl, 2-hydroxy-3-butenyl- and 4-hydroxybenzyl-glucosinolates. A collaborative study with Prof. B. Mitchell (University of Alberta) showed that the insect's primary receptors are located in the tarcae of the legs.

The flea beetle *Phyllotreta cruciferae* is differently attracted by different cruciferous plants with canola (*Brassica napus*) being highly attractive to this pest. Allyl isothiocyanate, a known attractant in many crucifers, is not found in canola. We are measuring correlation of content of canola compounds with attractiveness to beetles using several species.

The wheat midge (*Sitodiplosis mossellana*)

strongly prefers to lay its eggs in emerged, non-flowering wheat heads free of eggs. Collaborators (P. Hucl, Saskatchewan Wheat Pool and T. Wright, Agriculture Canada) screened 13 wheat varieties, and found three had some resistance to midge. No chemical attractants have been found.

5.2.3.2 Biochemistry

Plant lipases

The current and primary objective of this group is to isolate and characterize lipases from rapeseed (*Brassica napus*). PBI initiated this work in 1987 as part of a two-year joint venture agreement involving PBI, NRC's Biotechnology Research Institute (Montreal) and Biotechnica Canada Inc. (Calgary).

Acylglycerol acylhydrolases (lipases) from microbial sources have long been important in many chemical, industrial and pharmaceutical industries. However, there is a need for lipases having a well defined specificity, and improved thermal and organic solvent stability. Plant lipases are a virtually unknown quantity but the diversity of fatty acid and triglyceride compositions found in plants suggests that here is a likely source of lipases having properties important for industrial applications.

Plant lipase enzymes are membrane-associated. We have developed techniques for membrane release, for assays of activity and for characterization of some of these enzymes. Details of this work cannot be disclosed at this time because of proprietary considerations.

Modification of plant glucosinolate compositions

The objective of this work is to develop new methods for control and suppression of biosynthetic pathways in plants; the system being examined is mustard oil (glucosinolate) production in *Brassica* and related cruciferous species. These glucosinolates depress the quality of *Brassica* products, so

that their suppression or elimination in the plant is economically important. Several industrial firms examined the activities and found them to be consonant with their research needs and interests.

More than one hundred glucosinolate compounds are known to occur in plants, and their mode of biosynthesis is well documented. Using intact plants and *in vitro* plant cell cultures we found that the number of enzymes involved in the formation of this entire class of compound is considerably less than anticipated. In fact two key enzymes are utilized in synthesis of all glucosinolates. This finding permits the production of genetically engineered metabolic regulators (antisense RNA) which will prevent formation of the glucosinolates.

We developed rapid analyses for glucosyltransferase and sulfotransferase activities. Comparisons of these enzyme activities in high- and low-glucosinolate *Brassica* lines showed insignificant differences.

In the molecular biology part of the work, we constructed a *B. napus* cDNA library in phage lambda gt11, and obtained clones. The construction of other libraries is underway. Further work on suppression of glucosinolate enzyme activities has gone forward rapidly, the process being the subject of a patent application. We believe that genetic engineering of glucosinolate-free plants is now feasible. In preparation for upcoming experiments with *Brassica*, we studied the biosynthetic steps which might be most appropriately blocked by a study of mutants of *Arabidopsis thaliana*. Preliminary results have not been entirely clear-cut but we have established glucosinolate mutants.

5.2.3.3 Molecular Spectroscopy

The nuclear magnetic resonance instruments at PBI are a Varian 90 MHz and a Bruker AM-360 MHz (wide bore), of which the latter reported 1220 experimental uses in 1987/88. These included 1052



Technical Officer, Doug Olson, doing a single ion trace looking for specific compounds on a GC-MS run.

proton analyses, 84 carbon spectra and 84 phosphorus spectra.

The two mass spectrometers at PBI at the beginning of the year, Finnigan models 3300 and 4000 interfaced to a single data acquisition system, are old instruments. They logged 1065 experiments through the year. In January 1988 we received a new VG high resolution mass spectrometer with MS/MS which makes a variety of new and sophisticated biotechnological analyses possible. Like its predecessors, this instrument is available to extramural researchers on a fee-for-service basis.

5.3 Plant Cell Metabolism Project

5.3.1 Project Personnel

Research Officers

W. G.W.Kurz (leader), J. J. Balsevich, V. DeLuca, R. T. Tyler

Technical Officers

G. Bishop, K.B. Chatson, D. Dymond, J. Magus, B.D. Panchuk

Research Associates

S. Chevadej, N. Paiva, I. Roewer

Guest Researchers

U. Ellert, J. Alvarez Fernandez, R. Power, C. Rijnders, L. Tolonen

Summer Assistants

F. Chan, C. McNabb

5.3.2 Objectives and Background

The objectives of this project are to develop new methods for controlling metabolic pathways in plant cells, and to develop techniques for economic production of phytochemicals by *in vitro* cell processes.

5.3.3 Research Progress in 1987/88

This project has completed its fifth year and has established an array of basic technologies for large-scale growth of plant cells, for selection of lines and analysis of products, and for enhancement of secondary metabolite production. Many papers and patents have been generated. During 1987/88 the

group led an NRC interdivisional effort to evaluate new bioreactor designs, and continued earlier studies on elicitors, on chemistry of indole alkaloids and on gene expression regulation of biosynthetic steps.

Fungal elicitation of secondary metabolites

Extracts from some fungi, when added to plant cell cultures, elicit increased secondary metabolism. Process applications of this effect to metabolite production were patented by the project in 1986/87. This year we refined and improved this technology. Studies on elicitation of cell cultures focused on characterization of elicitors from *Botrytis* fungi for development of more predictable and reproducible elicitation systems.

Comparisons of fungal homogenate, mycelium and supernatant fractions revealed that elicitor activity was primarily associated with mycelium cell walls. Acid hydrolysates of cell wall material not only induced benzophenanthridine synthesis but did so without inducing a "browning" reaction. Thereafter, cell viability was easily maintained in elicited cultures enabling stronger elicitation – and therefore higher alkaloid yields along with easier sub-culture and re-elicitation. Unfortunately, production of this "non-browning" elicitor proved to be unpredictable, hydrolysates showing variable activity when produced under seemingly identical conditions.

Studies on characterization of the *Botrytis* elicitor and on alkaloid production in elicited, regenerating cells will be completed in 1988.

Phytochemistry of indole alkaloids

The medicinal plant *Catharanthus roseus* is the usual source of the anticancer drugs vinblastine and vincristine, the antihypertensive drug ajmalicine, and the alkaloids catharanthine and vindoline which are used as the feedstock for producing semi-synthetic anticancer drugs. The short term

goal of our work has been to develop a detailed understanding of the biosynthesis of alkaloids in this plant with a view to imposing gene expression controls.

Previous research showed the various steps in the vindoline pathway, and developed the enzymology of that pathway. Our 1987/88 studies on the regulation of pathways were based on this foundation. The effort has already produced a new, simple and inexpensive method for the extraction and isolation of plant alkaloids on a large scale. Also, PBI researchers found a very high alkaloid yielding plant line.

In this variant plant, vindoline biosynthesis is "turned off". An assay was developed and used to determine the distribution of alkaloids in mutant and normal plants. Leaf, vindoline concentrations were fairly constant in normal plants (400-600 mg/g) and did not vary greatly over time, but the variant exhibited extreme fluctuations (20-2500 mg/g) in vindoline content with time. Also it consistently produced novel minor alkaloids. One of these is a vindoline derivative with an unidentified substituent in the aromatic ring. Two cell lines have been initiated from the variant, from stem and leaf explants, for comparison of their alkaloid production with that of cell suspensions derived from normal plants.

Two in-house collaborations were initiated during the year. One involves the use of fast atom bombardment mass spectrometry for single-leaf analysis of indole alkaloids and glucosides; the other involves identification of biotransformation products from *Brassica* cell suspensions producing novel glucosinolates.

Enzymology of indole alkaloids

We demonstrated the last six steps of the pathway from tabersonine to vindoline (Fig. 5). This pathway was more complex than anticipated due to tissue-specific expression and extensive compartmental-

ization. The lack of expression of both N-methyltransferase and O-methyltransferase in cell suspension cultures and the possible need for light to "turn-on" O-acetyltransferase and to bring N-methyltransferase into developing thylakoids indicated the need of better understanding of differentiation. Thesis work of R. Power resulted in the purification of the O-acetyltransferase. The cloning of the structural gene for this protein for studying gene expression regulation is now possible.

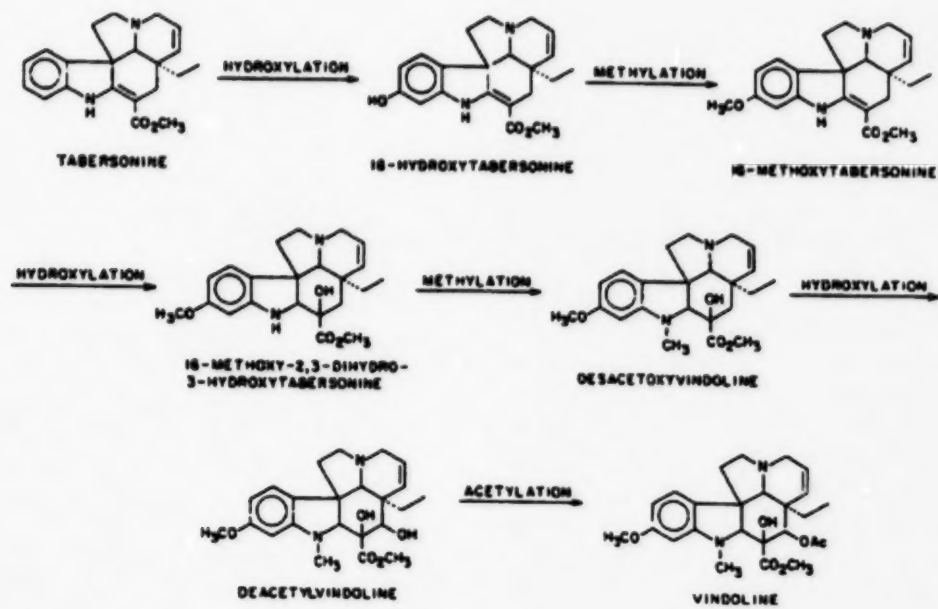
Total RNA was isolated from young 6.5-day-old seedlings which had been previously treated with 18 h of continuous light. Poly-A RNA was selected using oligo dT-cellulose chromatography. A cDNA library was constructed from poly-A-enriched mRNA, and packaged into a suitable bacterial host. After amplification, the library was screened for the presence of clones for tryptophan decarboxylase and acetylcoenzyme A-deacetyl-vindoline-O-acetyltransferase. We have now cloned DNA for both enzymes and have obtained inserts of 1800 and 1500 base pairs which should code for proteins of 50-60 and 40-50 kilodaltons. We are now sequencing these two clones.

Large-scale culture of plant cells

Aided by "Comprehensive Program" funding, the project began a two-year collaboration with the Biotechnology Research Institute (Montreal) to evaluate new types of bioreactors for large-scale growth of plant cell cultures. PBI's role is to optimize the surface immobilization characteristics of cells for metabolite production, while BRI's work involves the control and use of immobilized cells in bench scale and pilot scale reactors. The two PBI cell lines being used are poppy (*Papaver somniferum*, #2009) and periwinkle (*Catharanthus roseus*, #615).

Modification of some reactor design parameters, and optimization of hormone levels in media, allowed dry-weight cell densities of up to 150 g/m² to be handled, with vigorous production of alkaloids in small bioreactors.

Figure 5



Enzymology of indole alkaloids

6. Business and Outreach Activities

6.1 Overview

The Plant Biotechnology Institute carries out a series of nation-wide networking activities which comprise a program of "business and outreach". The main business activities are:

- General industrial liaison via the post of Industrial Liaison Officer;
- Contracting-out of parts of PBI's project plans through the Institute's Plant Biotechnology Fund;
- Joint venturing with external partners;
- Patenting and licensing of new PBI technology;
- Provision of research facilities and services;

- Collaborations and joint ventures with client partners.

Outreach activities ensure that the facilities and expertise of the Institute are known and readily accessible by researchers all over Canada. Among these activities are:

- Guest Researcher program;
- Affiliated Scientists program;
- Special events, including workshops and seminar series;
- Outreach publications, such as the PBI Bulletin.

Dr. Wolf Kurz (left), International Liaison Officer and head of the Biotech Development Office, discusses current technology transfer projects with Dr. Fred Constabel, Assistant Director of PBI.



6.2 Business Activities

6.2.1 Industrial Liaison

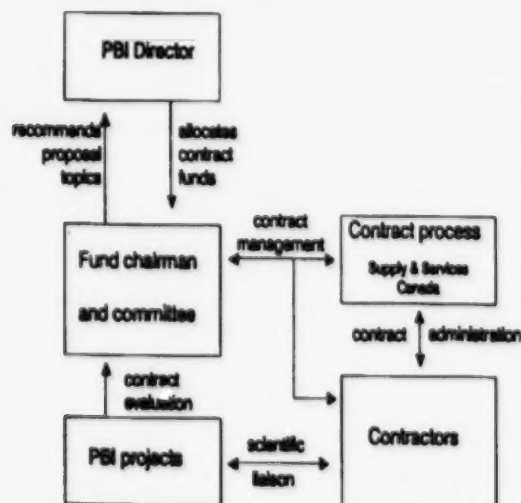
Dr. Louise Nelson
Industrial Liaison Officer

Much of the industrial liaison process is still the responsibility of each Institute project group. The setting-up in 1986 of an Industry Liaison Office with a named scientist-in-charge has provided a contact point for industries, added skills in patenting, licensing and negotiation of research Agreements, and a link for PBI with the nation-wide network of industrial liaison personnel.

6.2.2 Contracting-out: The Plant Biotechnology Fund

Dr. W.G.W. Kurz, Chairman
J.S. Katz, Fund Secretary

Nearly one-quarter of PBI's 1987/88 research operations were contracted out. Through our Plant Biotechnology Fund, we buy from other Canadian laboratories parts of our project work when we believe these parts can be performed better or quicker than would be possible in-house. Competitive selection is used in awarding contracts.



The Fund organization is shown below, along with contracts performed for PBI during the year.

The following contracts were performed in the budget year:

Horticultural Science and Plant Biotechnology Centre, University of Guelph, Guelph, Ontario (\$58 900), *Effect of nitrate on carbon and nitrogen partitioning in selected legumes*

Departments of Biology and Chemistry, University of Calgary, Calgary, Alberta (\$52 000), *Nitrogen metabolism in conifer tissues in vitro - Phase II*

Department of Biology, University of Calgary, Calgary, Alberta (\$47 000), *Comparison of gene expression between shoot-forming and non shoot-forming conifer cultures - Phase II*

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia (\$29 000), *Protection of Lodgepole pine from the mountain pine beetle*

Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan (\$56 250), *Biological screening of abscisic acid analogues which impart stress resistance to drought, salinity, freezing, and heat in cultivated crops*

Biology Department, Concordia University, Montreal, Quebec (\$25 474), *Localization of vindoline in Catharanthus roseus plants using immunological and immunocytological techniques*

Department of Plant Science, University of British Columbia, Vancouver, British Columbia (\$40 000), *Possible use of a bacterium causing gall formation on Douglas fir as an alternative transformation agent*

Department of Biology, University of Calgary, Calgary, Alberta (\$78 700), *Isolation of herbicide resistance genes from Brassica napus*

Department of Biology, University of Regina, Regina, Saskatchewan (\$62 200), *The identification and isolation of nuclear plasmids in Brassica species.*

6.2.3 Joint Venturing with External Partners

During 1987/88, PBI developed and signed four major new joint venture Agreements with external organizations. Our Agreement with the Saskatchewan Crop Development Center provides fuller access to the agronomic testing facilities of that organization, for laboratory strains of plants developed by means other than DNA recombinant techniques. It also reinforces existing collaborations between the two organizations.

A major joint venture involving PBI, the Biotechnology Research Institute (Montreal) and Biotechnica Canada Inc (Calgary) got underway early in the year. The goal of this 1988-1990 collaboration is development of new technology for metabolic modifications in plants, especially of lipids. Joint work begun in 1987 will extend to at least 1990.

The Saskatchewan Wheat Pool (Regina) and PBI, assisted by the federal Industrial Research Assistance Program, began a four-year joint research project on biotechnologies for improvement of wheat. Among the interests of this project are enhanced cold tolerance, plant regeneration from selected cells, and critical assessment of biotechnologically produced specimens.

Early in 1988 an important two-year Agreement was concluded with Vipont Research Laboratories Inc. (Fort Collins, Colorado) for scale-up of a cell metabolic process for production of the therapeutic alkaloid sanguinarine. Under the terms, a research team supported by Vipont will be located at PBI.

In addition to these major jointly-funded projects, the Institute carried out a large number of smaller collaborative projects.

PBI's active research collaborators during 1987/88: Starred (*) organizations have been involved in more than one project with PBI

ABL Forest Science Inc.
Agricultural Development Fund (Saskatchewan)
Agriculture Canada (Research Branch)*
AgriForest Technologies Ltd.
Allelix Inc.
Balco Reforestation Centre
Biotechnica Canada Inc.*
Biotechnology Research Institute (NRC)*
Canadian Forestry Service
Carleton University
CSIRO (Australia)
CSP Foods Ltd.
Division of Biological Sciences (NRC)
Esso Chemicals Inc.
McGill University
Michigan State University
Monsanto Inc.
National Research Council of Italy
Ontario Ministry of Agriculture and Food
Phero Tech Inc.
Philom Bios Inc.
Saskatchewan Crop Development Center
Saskatchewan Wheat Pool*
Simon Fraser University
Strawberry Ranch Inc.
Terochem Laboratories Inc.
Universidade federal do Parana (Brazil)
Université de Montréal
Université Laval
University of Alberta*
University of Braunschweig (Germany)
University of British Columbia
University of Calgary*
University of Guelph
University of Leiden (Netherlands)
University of Manitoba
University of Regina

University of Saskatchewan*
University of Toronto
UN Food and Agriculture Organization
Veterinary Infectious Disease Organization
Vipont Research Laboratories Inc.

6.2.4 Services to Canadian Research

These constitute an important element of the Institute's business program. Such services typically took three forms in 1987/88:

Services provided for fees. Notwithstanding the age of the mass spectrometry facilities which existed at PBI until February 1988, this analytical service led the fee-earners during the year, thanks to the skill of the system operators. Analyses by nuclear magnetic resonance, services based on the Institute's computer facilities, sale of research materials to on-site work groups, sale of a laboratory manual (Plant Cell Culture Techniques), and use of specialized instruments and machinery also generated revenue during the year.

Services provided without fees. As in previous years this amounted to more than 6000 hours of staff time, most of it from Ph.D. level personnel. The services rendered included:

- Training of industrial and international guests accepted in PBI's laboratories for that purpose;
- Expert review and comment on funding proposals for the Natural Sciences and Engineering Research Council (NSERC), the Medical Research Council, the Canadian Agricultural Research Council, the International Development Research Council, NRC Biotechnology reserve program, the federal Industrial Research Assistance Program, three federal departments, R&D agencies of the provinces of British Columbia, Alberta, Saskatchewan, Ontario and Quebec, and other bodies. PBI researchers reviewed an estimated 120 proposals;

- Advice and consultation via boards, committees, or directly through presentations or consultancies on request. In this context, note that PBI staff do not sit on boards or in management positions of commercial organizations and in all matters are strictly bound to respect the confidentiality of the bodies advised as well as of any information held in confidence by the Plant Biotechnology Institute. In all consulting, PBI staff act as agents of NRC and not in an individual capacity; they are bound by the National Research Council's conflict-of-interest controls;

- Refereeing of scientific publications. One quarter of PBI's Research Officers are members of editorial boards of scientific journals, and all staff refereed manuscripts during the past year. They carried out an estimated 400 such reviews.

Sales and loans of equipment by PBI to other research groups. During 1987/88 more than a dozen loan agreements were in force covering equipment valued at more than \$70 000.

Communication of results of open research is a principal part of the Institute's business. By such communication, the research results and directions of the Institute are made known, and external organizations are given the opportunity to benefit from basic new discoveries. During 1987 staff members authored or co-authored 78 publications in scientific journals and books, and presented 34 papers at meetings around the world describing NRC's research advances in plant biotechnology. Presentations were made in and beyond Canada, and as usual a large number involved collaborative work with non-NRC scientists in different parts of the country. Chapter 8 of this Annual Report lists the papers emanating from PBI during the year.

6.2.5 Patents from the Institute

Patents in force at 31 March 1988 are listed below. PBI researchers applied for new patents on three novel processes relating to DNA recombinant technology, including the first series of NRC proprietary genes from PBI, and on a set of new plant growth regulator chemicals. These cases were in process at year-end.

Continuous Fermentation with Phased Growth
(P.S.S. Dawson)

United States Patent No. 3,647,633

Treatment of Rapeseed Meal
(C.G. Youngs, H.R. Sallans, J.M. Bell)

Canadian Patent No. 839,653;

United States Patent No. 3,560,217

Dome Fermentor
(M.S. Anderson and others)

United States Patent No. 3,713,988;

Canadian Patent No. 929,121

Texturing Process for Vegetal Protein
(A.K. Sumner, C.G. Youngs, L. Leinan)

Canadian Patent No. 1,001,890;

United States Patent No. 3,969,539

Viscosity Measuring Apparatus
(A.A. Sieben)

United States Patent No. 3,983,731;

Canadian Patent No. 1,005,564

Colorimetric Assay for Nitrogenase
(T.A. Larue, W.G.W. Kurz)

Canadian Patent No. 1,012,449;

United States Patent No. 3,904,481

High Protein Snack Foods
(B.D. Panchuk, M. Anderson, C.G. Youngs)

Canadian Patent No. 1,021,994;

United States Pat. No. 4,140,803

Composite Attractant for Bertha Armyworm Moth
(E.W. Underhill and others)

United States Patent No. 4,042,681;

Canadian Patent No. 1,059,023

Attractant for Sunflower Moth
(E.W. Underhill)

United States Patent No. 4,284,622;

Canadian Patent No. 1,139,659

Producing Codeine from Cell Cultures
(F. Constabel, W.G.W. Kurz, W.H.J. Tam)

Canadian Patent No. 1,134,764;

Patents in other countries

Attractant for Tent Caterpillar Moth
(W.F. Steck and others)

Canadian Patent No. 1,212,697

Detoxification of Tannin-containing Seeds
(R.D. Reichert, D.J. Schwab)

Canadian Patent No. 1,190,085

RNA Plant Virus Vectors
(L.E. Pelcher, M.C. Halasa)

Canadian Patent No. 1,191,510;

Modular Stackable Cone Orifice Type Insect Trap
(W.F. Steck and others)

Canadian Patent No. 1,157,645;

United States Patent No. 4,452,006

Multicomponent Attractant for Armyworm Moths
(W.F. Steck and others)

Canadian Patent No. 1,172,163;

United States Patent No. 4,575,458

Attractants for Fall Cankerworm Moths
(E.W. Underhill and others)

Canadian Patent No. 1,231,982;

United States Patent No. 4,610,876

6.3 Outreach Activities

The Plant Biotechnology Institute is a national laboratory, serving all parts of Canada. For this role we have set up an array of outreach initiatives over the past three years; and have brought the system to completion in 1987/88.

6.3.1 The Guest Researcher Program

This remained the cornerstone of PBI's outreach program. Under the 1987/88 program, 93 qualified extramural scientists accessed the Institute's entire facilities for agreed research, development or training activities. Personnel from Canadian industries and Canadian universities led the list of those sponsoring guests at the Institute; other sponsors included public agencies, co-operatives, international organizations and foreign research institutions (frequently under bilateral scientific agreements). NRC paid no salary or stipends to guests but did provide basic research accommodation, materials and such supervision as was necessary, usually without charge. Guest Researchers during 1987/88 are named at the beginning of this Report.

We improved the program in two ways during 1987. First, we made travel and living assistance available to selected students from graduate programs of Canadian universities who came to the Institute to carry out some or all of their thesis research. Commitment, agreement and close co-operation among student, the university and the Institute remained vital elements for the success of this program. Now for the first time, students from universities distant from Saskatoon access PBI without a "penalty" of heavy travel costs. Second, the Institute constructed more and better physical accommodation for guests on the premises. One centre for guests in the Biological Chemistry Laboratory was geared for 10 users, while smaller new space in the Cell Technology Laboratory accommodated 6 new arrivals.

6.3.2 The Affiliated Scientists Program

During 1987/88, this new program expanded to bring seven outstanding Canadian biotechnologists into formal association with PBI. This recognition of exceptional achievers in plant science is helping solidify informal contacts and provides an avenue for more expert discussion in the national plant biotechnology effort. Our Affiliates are listed in Chapter 2 of this Report.

6.3.3 Special Events and Seminar Series

Wheat and Barley Biotechnology Days (11-12 June 1987) brought 105 leading researchers to PBI to discuss the state of the art. PBI sponsored five smaller biotechnology information workshops through the year.

Several important federal biotechnology planning meetings took place at PBI, including two meetings of the Institute's Board of Governors. The Canadian Agricultural Research Council's standing committee on biotechnology in food and agriculture held its main 1987 meeting at the Institute (12 November), and six other biotech groups held meetings on our premises this year.

The Minister of State for Science and Technology, the Honourable Frank Oberle, visited the Institute on 12 May 1987. PBI staff conducted twenty-nine other tours for distinguished visiting groups.

A major information inflow effort by PBI resulted in a large number of invited seminar visitors. Details of the main seminar series follow on the next pages. An estimated 2800 people from throughout western Canada attended PBI-sponsored seminars during the year.

PBI's Main Seminar Series, 1987/88

1987

April 1

Dr. J.L. Bennetzen

Purdue University

West Lafayette, Indiana

The "mutator" transposable element system of maize

April 7

Dr. Bill Crosby

Plant Biotechnology Institute

Overview of gene patenting conference

April 8

Dr. Roland Brousseau

Biotechnology Research Institute

Montreal, Quebec

Molecular genetics of delta endotoxin production in Bacillus thuringiensis

April 22

Dr. Roxanne Deslauriers

NRC, Biological Sciences

Ottawa, Ontario

Application of nuclear magnetic resonance spectroscopy

May 8

Dr. Andrew D. Hanson

Michigan State University

East Lansing, Michigan

Finding and using genes for environmental stress

May 28

Dr. G. Fletcher

Marine Sciences Lab

Memorial University

Why don't arctic fish freeze during winter?

June 1

Dr. Lesh Klimczak

Plant Biotechnology Institute

Plant protein kinases - in search of regulatory elements

June 29

Dr. K. Kartha

Plant Biotechnology Institute

Highlights of the Gordon Research Conference on plant cell and tissue culture

August 7

Dr. D. Werner

Universität Marburg

Federal Republic of Germany

Plant and microbial symbioses

August 25

Dr. Antoni Slabas

Unilever Research, U.K.

Unravelling the enzymes of lipid biosynthesis in plants

September 1

Dr. G.E. Templeton

University of Arkansas

Fayetteville, Arkansas

Commercialization of an indigenous fungus for the "Collego" mycoherbicide

September 2

Dr. K. Ohyama

Kyoto University

Kyoto, Japan

Complete nucleotide sequence of chloroplast from a liverwort, Marchantia polymorpha: Gene organization and new group of genes

October 7

Dr. David Cass

University of Alberta, Edmonton

Gamete fusion in angiosperms

October 21

Dr. Anthony Huang

University of South Carolina

Cell and molecular biology of lipid bodies in seeds

October 29

Dr. John Mahon

Plant Biotechnology Institute

Can the primary structure of RUBISCO Large Subunit account for differences in its catalytic properties?

November 5

Dr. Pal Maliga

Advanced Genetic Sciences Inc.

Oakland, California

Chloroplast recombination in Nicotiana

November 13

Dr. John Carlson

Allelix Inc.

Mississauga, Ontario

Crop transformation projects at Allelix

November 18

Dr. John P. Ohlrogge

Michigan State University

East Lansing, Michigan

Plant acyl carrier proteins

December 7

Dr. Wilf Keller

Agriculture Canada

Ottawa, Ontario

An overview of plant biotechnology research at the Plant Research Centre, Ottawa

December 9

Dr. James Procunier

University of Western Ontario

London, Ontario

Mammalian cancer genes and "proto-onco genes in plants"

December 14

Dr. Ron Sederoff

USDA Forest Service

Berkeley, California

Genetic manipulation in gymnosperms and angiosperms - a progress report

December 16

Dr. Janet Taylor

Max-Planck Institute

Koln-Vogelsang, Germany

Isolation and characterization of defense-related genes in potato

December 17

Dr. Mohammed Saleem

Plant Biotechnology Institute

7th International Protoplast Symposium: a trip report

1988

January 11

Dr. Terry J. Reisch

Agriculture Canada

Ottawa, Ontario

Development of micro-injection technology for crop plants

January 13

Dr. Vincenzo DeLuca

Plant Biotechnology Institute

Regulation of monoterpenoid indole alkaloid biosynthesis in Catharanthus roseus

January 20

Dr. Keith Downey

Agriculture Canada

Saskatoon, Saskatchewan

Oilseed Brassica - the oil crops of today and tomorrow

January 27

Dr. W. L. Crosby

Plant Biotechnology Institute

Genetic transformation of Brassica napus

January 29

Dr. R. D. Hill

University of Manitoba

Winnipeg, Manitoba

Hormonal regulation of protein synthesis in cereal α-luron layers

February 8

Dr. J. S. Rokem
The Hebrew University
Jerusalem, Israel

Secondary metabolite formation (diosgenin and phaseollin) in plant cell tissue culture

February 17

Dr. R. Chibbar
Plant Biotechnology Institute

Somatic embryogenesis - a model system to study plant development

February 24

Dr. Eleanor White
Canadian Forestry Service
Victoria, B.C.

Integration of molecular biology with classical tree breeding

March 9

Dr. William L. Ogren
U.S. Dept. of Agriculture
Urbana, Illinois

*RUBISCO activase regulator of RuBP carboxylase/oxygenase activity *in vivo**

March 23

Dr. John Vederas
Department of Chemistry
University of Alberta, Edmonton

Unusual amino acids: synthesis, enzyme inhibition and biological activity

researchers to better access the Institute's resources.

Besides regular publications, individual responses to more than 400 inquiries were sent out, many to newspaper and telemedia people covering biotechnology, agriculture or forestry news. As a result, PBI scientists gave local and national radio and television interviews and six new articles about the Institute appeared in major publications:

- PBI Seeds a New Idea, *New Biotech* April 1987 (page 10)
- Cloning New Foods, *Maclean's* magazine 20 April 1987 (page 43)
- Les Gros et Les Petits, *Québec Science* May 1987 (pages 40-46)
- The Plant Biotechnology Institute, *Biotechnology Bulletin* February 1988 (pages 1-3)
- Biotech Route to Dental Drug, *Chemical Marketing Reporter* (New York, N.Y.) 2 March 1988
- Colo., Cdn Firm Sign R & D Pact, *Journal of Commerce* 2 March 1988

6.3.4 Outreach Publications

To communicate news of the Institute's work to all parts of Canada and beyond, the 1986/87 Annual Report appeared in August, while the PBI Bulletin reached more than 900 firms and laboratories at three-month intervals. Two popular new features of the Bulletin have been inclusion of news from other Canadian plant biotechnology centres and "profiles" on these centres. A description of the physical facilities available for research at PBI helped many

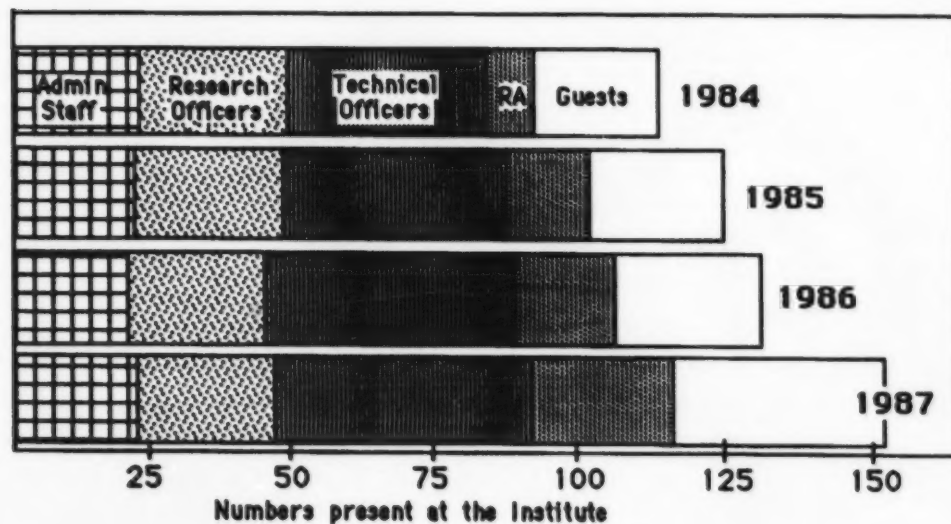
7. Support Services

7.1 Overview

The Support Services Section helps provide for the day-to-day operational needs of the Institute. As a publicly-owned corporation, the National Research Council is required to maintain certain standards and to administer certain programs in accordance with the policies of the Government of Canada. For example, handling of public records -- that is, almost all the documents and other written material dealt with by the Institute -- must be done in conformity with NRC standards and with such legislation as the Privacy Act and the Access to Information

Act. Similarly, the administration of financial affairs must satisfy strict requirements of public accountability. The Section carries out these duties while providing numerous scientific and technical services to the research projects.

A unique feature of PBI is the existence of user/supplier joint committees which ensure that the services being offered do indeed match the current needs of users in the Institute's laboratories.



7.2 Services

7.2.1 Administrative Services

Personnel:

Joan L. Yorke (Administrative Officer), Cheryl Bitz, Rosemarie Gallays, Dawn Larson, Kathy Seabrook, Kathy Wilson, Ed Knapp (Research Illustration)

Administrative Services has charge of personnel and guest programs, finance, security, reception, internal communications, manuscript and poster preparation, records management, telecommunications and office systems. In 1987/88, some streamlining of the Service occurred, and during the year the research illustration office was merged into the unit.

7.2.2 General Services

Personnel:

T. Martin Mallard (Superintendent of Services), Bill Brumwell, Armand Fendelet, Harry Holfeld, Rick McDonald, George Reynolds, Wayne Stankewich

General Services provides, on one hand, upkeep and updating of the electronic/computer systems used in most parts of the building; and on the other, the management of all building services including engineering and maintenance, mechanical and electrical services, renovations, new construction, water, heat, light, storage, waste disposal systems and machine shop services.

The year 1987/88 saw an unusually large number of building alterations carried out to allow new projects to go ahead. A new centre for guest researchers accommodates ten or more people in pleasant off-lab space. Two new laboratory units for plant cell technology research were brought onstream,

one being created from space previously used as a vehicle garage. The most ambitious renovation of the year was the conversion of a former unit operations area into a large new site for the Plant Biotechnology Branch of the Canada Institute for Scientific and Technical Information (CISTI). This library and information centre, completed in March 1988, is a joint undertaking of CISTI and PBI.

7.2.3 Supply Services

Personnel:

W. S. Wiley (Supply Officer), Olga Coates, Jill Horn, Terry Mazurkevich, James McHarg, Irene Philipchuk

Supply Services oversees the ordering and delivery of all PBI's purchases. Its responsibilities include tendering, shipping and receiving, on-site storage and distribution of goods, capital inventory, management of PBI's motor vehicles. The unit is the Institute's link with the NRC financial management network.

7.3 Committees

Four user/supplier committees were active during 1987/88.

- The **Computer Committee** advised on equipment purchases and on future kinds and levels of use of central and micro units at PBI. Members are: M. Mallard (chair), D. Guenter (IRC), C. Bock, D. Tenaschuk, H. Holfeld.

- The **Special Events Committee** organized several events presenting the Institute to visitors. These included tours for the Standing Committee on Science and Technology from the House of Commons, the Ambassador of Egypt, Chinese delegations, Italian attache, Ambassador of Pakistan, members of the Canadian High Commission, Board of Governors, Wheat Pool, and other business groups. Members for 1987 were L. Blashill (chair), D. Kneeshaw, T. Bethune, G. Bishop.

- The **Library Services Committee** assists CISTI in choosing its journal and book orders to match project interests and needs, and provides the library with information and evaluations of current holdings and services. Members are F. Chen (chair), L. Pelcher, A. Cutler, R. Weselake.

- NRC's **Interdivisional Committee on Safety and Health** continued to be an important and effective body for advising Administration of staff concerns in these areas, and for promoting safe work practices throughout the Institute. The group has responsibilities in hazardous chemical procedures and in first aid training for staff. Members are: J. Magus (co-chair), B. Reichert (co-chair), B. Chatson, J. Hammerlindl, D. Horn, S. Hobbs, L. Pelcher, J. Yorke.

Two research program committees continued to operate throughout the year to facilitate inter-project planning, financing of work, contracting-out and the coordination of ongoing research:

- The **Planning and Review Committee**, consisting of PBI Management and project leaders, met sixteen times and remained the Institute's primary means of program integration and development. Members are: W. F. Steck (chair), F. Constabel, W.L. Crosby, D. I. Dunstan, K. K. Kartha, W.G. W. Kurz, J. D. Mahon, E. W. Underhill, C. Walker (secretary) and J. L. Yorke.

- The **Plant Biotechnology Fund Committee** assessed contract proposals for 1988 and reviewed contract performance from last year. It recommended significant changes in the procedures for the Fund which were implemented this year. Members are: W.G.W. Kurz (chair), F. Constabel, W. L. Crosby, D. I. Dunstan, K. K. Kartha, J. S. Katz (secretary), J. D. Mahon, E. W. Underhill.

8. Publications and Presented Papers, 1987

1. Abrams, S.R. and A.C. Shaw. 1987. **On the mechanism of 1,3-prototropic shifts in acetylene-allene isomerizations.** Journal of Organic Chemistry 52: 1835-1839. NRCC No. 27511.
2. Abrams, S.R. and A.C. Shaw. 1987. **Triple bond investigations: 2- to 9-decyn-1-ol.** Organic Syntheses 66: 127-131.
3. Abrams, S.R. 1987. **Direct deuterium labelling of xanthone with the lithium salt of 1,3-diaminopropane-N,N,N',N'-d4.** Journal of Labelled Compounds and Radiopharmaceuticals XXIV: 941-948.
4. Abrams, S.R. and A.C. Shaw. 1987. **Preparation of 8-methyl-2-decanol: general synthesis of diastereomeric mixtures of alkyl branched insect pheromones.** Journal of Chemical Ecology 13: 1927-1933. NRCC No. 27519.
5. Abrams, S.R., M. Green, R.P. Steer and M. Szmanski. 1987. **Unusual deuterium isotope effect on the radiationless decay of xanthone S₂.** Chemical Physics Letters. 139: 182-186.
6. Adams, R.P., A.L. Almirall and L. Hogge. 1987. **The volatile leaf oils of the junipers of Cuba: Juniperus lucayana Britton and Juniperus saxicola Britton and Wilson.** Flavour and Fragrance Journal 2: 33-36.
7. Archambault, J., B. Volesky and W.G.W. Kurz. 1987. **Development of bioreactors for the culture of immobilized plant cells.** Proceedings of 37th Canadian Chemical Engineering Conference 18-22 May, 1987. Montreal, Quebec, pp. 308-310.
8. Attree, S.M., F. Bekkaoui, D.I. Dunstan and L.C. Fowke. 1987. **Regeneration of somatic embryos from protoplasts isolated from an embryogenic suspension culture of white spruce (Picea glauca).** Plant Cell Reports 6:480-483.

9. Bekkaoul, F., P. Saxena, S.M. Attree, L.C. Fowke and D.I. Dunstan. 1987. **The isolation and culture of protoplasts from an embryogenic cell suspension culture of Picea glauca (Moench) Voss.** Plant Cell Reports 6: 476-479. NRCC No. 27937.
10. Chalifour, F.-P and L.M. Nelson. 1987. **Effects of continuous nitrogen supply on symbiotic dinitrogen fixation of faba bean and pea inoculated with different rhizobial isolates.** Canadian Journal of Botany 65: 2542-2548. NRCC No. 27515 .
11. Chen, T.H.H. and K.K. Kartha. 1987. **Cryopreservation of woody species.** In: Cell and Tissue Culture in Forestry, Vol. 2, J.M Bonga and D.J. Durzan (eds.), Martinus Nijhoff Publishers, Dordrecht/Boston/Lancaster, pp. 305-319.
12. Chen, T.H.H., M.D. Lazar, G.J. Scoles, L.V. Gusta and K.K. Kartha. 1987. **Somaclonal variation in a population of winter wheat.** Journal of Plant Physiology 130: 27-36. NRCC No. 27931.
13. Chibbar, R.N., R. Esnault, A. Brickenden, J. Dyck and R.B. van Huystee. 1987. **A highly specific monoclonal antibody to a cationic peroxidase from cultured peanut cells.** Journal of Plant Physiology 129: 103-110. NRCC No. 26777.
14. Constabel, F. 1987. **Cell culture in phytochemistry.** Cell Culture and Somatic Cell Genetics of Plants (ed. I.K. Vasil). Academic Press, Inc. 4: 3-13.
15. Cutler, A.J. and M. Saleem. 1987. **Permeabilizing soybean protoplasts to macromolecules using electroporation and hypotonic shock.** Plant Physiology 83: 24-28. NRCC No. 25552.
16. Cutler, A.J. and M. Saleem. 1987. **Effect of protoplasting stress on peroxidase and lipoxygenase activities in cereal protoplasts.** Plant Physiology 83:464.
17. Dawson, P.S.S. and J.C. MacDonald. 1987. **Changes in phosphorus magnetic resonance spectra during the cell cycle of phosphorus limited phased culture of Canadida utilis.** FEMS Microbiology Letters 43: 57-60. NRCC No. 27512.
18. DeLuca, V., A. Cutler, J. Balsevich and W.G.W. Kurz. 1987. **The compartmentation of the late stages of vindoline biosynthesis in Catharanthus roseus (periwinkle).** Plant Physiology 83 (4 suppl.):126.
19. DeLuca, V. and A.J. Cutler. 1987. **Subcellular localization of enzymes involved in indole alkaloid biosynthesis in Catharanthus roseus.** Plant Physiology 85: 1099-1102. NRCC No. 27932.

20. DeLuca, V., J. Balsevich, R.T. Tyler and W.G.W. Kurz. 1987. **Characterization of a novel N-methyltransferase (NMT) from intact Catharanthus roseus plants. Detection of NMT and other enzymes of the indole alkaloid biosynthetic pathway in different cell suspension culture systems.** Plant Cell Reports 6: 458-461.
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22. Dimmock, J.R., S.A. Patil, M.S. Sardesai and M. Mazurek. 1987. **Anticonvulsant evaluation of some 3-oxo and 3-thiosemicarbazono analogues of l-aryl-l-ethylthio-nonanes and related compounds.** Pharmazie 42: H.2. NRCC No.27932.
23. Dunstan, D.I., G.H. Mohammed and T.A. Thorpe. 1987. **Morphogenetic response of vegetative bud explants of adolescent and mature Picea glauca (Moench) Voss in vitro.** New Phytologist 106: 225-236. NRCC No. 25548.
24. Ehiwe, A.O.F., R.D. Reichert, D.J. Schwab, E.S. Humbert and G. Mazza. 1987. **Effect of seed moisture content and temperature on the seed coat durability of field pea.** Cereal Chemistry 64: 237-239. NRCC No. 27513.
25. Ehiwe, A.O.F. and R.D. Reichert. 1987. **Variability in dehulling quality of cowpea, pigeon pea, and mung bean cultivars determined with the tangential abrasive dehulling device.** Cereal Chemistry 64: 86-90. NRCC No. 26779.
26. Eilert, U., J. Balsevich and W.G.W. Kurz. 1987. **Greening and strictosidine lactam production in two cell lines of Vinca major cv. variegata.** Plant Cell, Tissue and Organ Culture 8: 177-182. NRCC No. 26184.
27. Eilert, U., W.G.W. Kurz and F. Constabel. 1987. **Alkaloid accumulation in plant cell cultures upon treatment with elicitors.** Plant Tissue and Cell Culture 1987: 213-219. Eds.: C.E. Green, D.A. Somers, W.P. Hackett and D.D. Biesboer; Alan R. Liss, Inc., New York. NRCC No. 26102.
28. Eilert, U., V. DeLuca, F. Constabel and W.G.W. Kurz. 1987. **Elicitor-mediated induction of tryptophan decarboxylase and strictosidine synthase activities in cell suspension cultures of Catharanthus roseus.** Archives of Biochemistry and Biophysics 254: 491-497. NRCC No. 26783.
29. Eilert, U., V. DeLuca, W.G.W. Kurz and F. Constabel. 1987. **Alkaloid formation by habituated and tumorous cell suspension cultures of Catharanthus roseus.** Plant Cell Reports 6: 271-274. NRCC No. 27514.
30. Eilert, U., W.G.W. Kurz and F. Constabel. 1987. **Ultrastructure of Catharanthus roseus cells cultured in vitro and exposed to conditions for alkaloid accumulation.** Protoplasma 140: 157-163. NRCC No. 24524.

31. Ellis, R.J., S.M. van der Vies and S.M. Hemmingsen. 1987. **The Rubisco large subunit binding protein - a molecular chaperone?** In *Plant Molecular Biology 1987*, (eds. von Wettstein, D. and Chua, N.-H.) NATO-ASI Series A. Copenhagen. Life Sciences 140: 33-40, Plenum Press, New York and London.
32. Esnault, R., R.N. Chibbar, D. Lee and R.B. van Huystee. 1987. **Early differences in production of mRNAs for phenylalanine ammonia-lyase and chalcone synthase in resistant and susceptible cultivars of soybean inoculated with Phytophthora megasperma f. sp. glycinea.** *Physiological and Molecular Plant Pathology* 30: 293-297.
33. Hahlbrock, K., C. Cretin, B. Cuypers, K.-H. Fritzenmeier, K.-D. Hauffe, W. Jahnen, E. Kombrink, F. Rohwer, D. Scheel, E. Schmeizer, M. Schröder and J. Taylor. 1987. In *"Plant Molecular Biology. NATO Advanced Science Institute Series."* Eds. von Wettstein, D. and N.-H. Chua. Plenum Press. New York and London. 399-406.
34. Hobbs, S.L.A. 1987. **Comparison of photosynthesis in normal and triazine-resistant Brassica.** *Canadian Journal of Plant Science* 67: 457-466. NRCC No. 25864.
35. Hogge, L.R. and J.G. Millar. 1987. **Characterization of unsaturated aliphatic compounds by GC/mass spectrometry.** *Advances in Chromatography* 27: 299-351. NRCC No. 27516.
36. Hu, C., H., D. Lee, R.N. Chibbar and R.B. van Huystee. 1987. **Ca²⁺ and peroxidase derived from cultured peanut cells.** *Physiologia Plantarum* 70: 99-102.
37. Hynes, R.K. 1987. **Use of liquid chromatography to measure chlorite production by Nitrobacter winogradskyi.** *FEMS Microbiology Letters* 41: 181-184. NRCC No. 26538.
38. Iacomini, M., A.M.W. Zanin, J.D. Fontana, L. Hogge and P.A.J. Gorin. 1987. **Isolation and characterization of β -D-glucan, heteropolysaccharide, and trehalose components of the basidiomycetous lichen Cora pavonia.** *Carbohydrate Research* 168: 55-65. NRCC No. 24320.
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41. Jain, J.C., P.D. Shargool and S. Chung. 1987. **Compartmentation studies on enzymes of ornithine biosynthesis in plant cells.** *Plant Science* 51: 17-20.
42. Kartha, K.K. 1987. **Advances in the cryopreservation technology of plant cells and organs.** *Plant Tissue and Cell Culture 1987*: 447-458. NRCC No. 26013.

43. Kartha, K.K. 1987. **Cryopreservation of secondary metabolite-producing plant cell cultures.** *Cell Culture and Somatic Cell Genetics of Plants* (ed. I.K. Vasil). Academic Press, Inc. 4: 217-227. NRCC No. 26776.
44. Kurz, W.G.W., F. Constabel, U. Eilert and R.T. Tyler. 1987. **Elicitor treatment: a method for metabolite production by plant cell cultures in vitro.** In: "Topics in Pharmaceutical Sciences" Eds.: Breimer, D.D. Speiser, P. Elsevier Science Publishers B.V. pp 283-290. NRCC No. 27518.
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46. Levadoux, W.L., M.L. Kalmokoff, M.D. Pickard and J.W.D. GrootWassink. 1987. **Pigment removal from canola using chlorophyllase.** *Journal of the American Oil Chemists' Society* 64: 139-144. NRCC No. 26537.
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49. MacDonald, J.C. and M. Mazurek. 1987. **Phosphorus magnetic resonance spectra of open-chain linear polyphosphates.** *Journal of Magnetic Resonance* 72: 48-60. NRCC No. 26778.
50. MacDonald, J.C. and C.A. Bock. 1987. **Spectral properties of pseudobactin produced by Pseudomonas sp. B10.** *Canadian Journal of Botany* 65: 703-705. NRCC No. 26780.
51. MacKenzie, S.L., D. Tenaschuk and G. Fortier. 1987. **Analysis of amino acids by gas-liquid chromatography as tert-butyldimethylsilyl derivatives. Preparation of derivatives in a single reaction.** *Journal of Chromatography* 387: 241-253. NRCC No. 26536.
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53. MacKenzie, S.L. 1987. **The heptafluorobutyryl isobutyl ester derivatives of amino acids. Derivatization, separation and applications.** In "Amino Acid Analysis by Gas Liquid Chromatography." (eds. Gehrke, G.W., Juo, K.C. and Zumwalt, R.W.) CRC Press, Inc. Boca Raton, U.S.A. pp. 93-117.

54. Mahon, J.D. and S.L.A. Hobbs. 1987. Genetic control of photosynthesis in relation to growth of pea (Pisum sativum L.) plants. In "Progress in Photosynthesis Research" (ed. J. Biggins). Martinus Nijhoff 4: 385-391. NRCC No. 26178.
55. Milar, J.G., E.W. Underhill, M. Giblin and D. Barton. 1987. Sex pheromone components of three species of Semiothisa (Geometridae): (Z,Z,Z) 3,6,9-heptadecatriene and two monooxydiene analogs. Journal of Chemical Ecology 13: 1371-1282. NRCC No. 26108.
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57. Nelson, L.M. 1987. Response of Rhizobium leguminosarum isolates to different forms of inorganic nitrogen during nodule development in pea (Pisum sativum L.). Soil Biology and Biochemistry 19: 759-763. NRCC No. 26785
58. Pivnick, K.A. and J.N. McNeil. 1987. Diel patterns of activity of Thymelicus lineola adults (Lepidoptera: Hesperidae) in relation to weather. Ecological Entomology 12: 197-207.
59. Pivnick, K.A. and J.N. McNeil. 1987. Puddling in butterflies: sodium affects reproductive success in Thymelicus lineola. Physiological Entomology 12: 461-472.
60. Reed, D.W., E.W. Underhill and E.M. Giblin. 1987. Attraction of sphingid moths (Lepidoptera: Sphingidae) to 10,12-hexadecadienyl aldehydes and acetates: evidence of pheromone components. Journal of Chemical Ecology 13: 931-942. NRCC No. 25857.
61. Reichert, R.D. and A.O.F. Ehlwe. 1987. Variability, heritability and physiochemical studies of seed coat durability in field pea. Canadian Journal of Plant Science 67: 667-674. NRCC No. 27520.
62. Saleem, M. and A.J. Cutler. 1987. Stabilizing corn leaf protoplasts with n-propyl gallate. Journal of Plant Physiology 128: 479-484. NRCC No. 26179.
63. Saleem, M. and A.J. Cutler. 1987. Uptake of macromolecules by osmotically permeabilized plant protoplasts. Plant Physiology 83: 1595.
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75. Underhill, E.W., C.E. Rogers and L.R. Hogge. 1987. Sex attractants for two sunflower pests, Eucosma wemonana (Lepidoptera: Tortricidae) and Leophrictis similis (Lepidoptera: Gelechiidae). *Environmental Entomology* 16: 463-466. NRCC No. 26532.
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77. von Rudloff, E. 1987. The volatile twig and leaf oil terpene compositions of three western north american larches. Larix laricina, Larix occidentalis, and Larix lyallii. *Journal of Natural Products* 50: 317-321. NRCC No. 25859.

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79. Xia, X., G. Selvaraj and H. Bertrand. 1987. **Molecular characterization of an unusual repetitive DNA from a cultivar of canola (*Brassica napus*).** Genetics Society of Canada Bulletin 18: 128.

Poster/Abstract Presentations

1. Abrams, S.R. and B.V. Milborrow. **Synthesis and biological activity of an abscisic acid analog.** Canadian Chemical Congress, Quebec City, June 8, 1987.
2. Abrams, S.R. and B.V. Milborrow. **Abscisic acid analogs.** 4th International Conference on Chemistry and Biotechnology of Biologically active Natural Products, Budapest, Hungary, August 12, 1987.
3. Attree, S.M., F. Bekkaoui, P.K. Saxena, D.I. Dunstan and L.C. Fowke. **Differences in developmental morphology of protoplasts from two cell lines of white spruce (*Picea glauca*).** 7th International Protoplasts Symposium, Wageningen, Holland, December 6-11, 1987.
4. Baillie, A.M.F., K.K. Kartha and B.G. Rossnagel. **A tissue culture regeneration system for barley (*Hordeum vulgare* L.).** 13th North American Barley Research Workshop, Montana State University, Bozeman, Montana, July 26-30, 1987.
5. Bhanot, P., F. Georges and E.B. Waygood. **Site-directed mutagenesis of the carboxyl terminus glutamate of HPr, a phosphocarrier protein of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) of *E. coli*.** Canadian Federation of Biological Societies, Winnipeg, Manitoba, June 22-26, 1987.
6. Chibbar, R.N., J. Shyluk, C. Mallard and F. Constabel. **Esterase isozymes as markers of somatic embryogenesis in cultured carrot cells.** Annual Meeting of the American Society of Plant Physiologists, St. Louis, Mo., U.S.A., July 19-23, 1987. Plant Physiology 83(4S): 76.
7. Chibbar, R.N., J. Shyluk, F. Georges and F. Constabel. **Biochemical aspects of sunflower protoplast culture.** Annual Meeting of the American Society of Plant Physiologists, St. Louis, Mo., U.S.A., July 19-23, 1987. Plant Physiology 83(4S): 74.
8. Chibbar, R.N., J. Shyluk, F. Georges and F. Constabel. **Role of proline in somatic embryogenesis in cultured carrot cells.** Annual Meeting of the American Society of Plant Physiologists, St. Louis, Mo., U.S.A., July 19-23, 1987. Plant Physiology 83(4S): 76.
9. Constabel, F. **Plant cell culture in phytochemistry.** Symposium on biotechnology, 14th International Botanical Congress, Berlin, July 27-31, 1987.

10. De Luca, V., A.J. Cutler, J. Balsevich and W.G.W. Kurz. **The compartmentation of the late stages of vindoline biosynthesis in Catharanthus roseus (Periwinkle).** Plant Physiology 83: 126S. Annual Meeting of the American Society of Plant Physiologists, St. Louis, Mo, July 19-23, 1987.
11. De Luca, V., J. Balsevich, R. Tyler and W.G.W. Kurz. **Isolation and characterization of a specific N-methyltransferase involved in indole alkaloid biosynthesis from Catharanthus roseus.** 27th Annual Meeting, The Phytochemical Society of North America, University of South Florida, Tampa, Florida, June, 1987.
12. Dunstan, D.I. **Tissue culture of Canadian conifers.** Canadian Plant Physiology Association (Technical Section Research Committee), Niagara-on-the-Lake, Ontario, October 19, 1987.
13. Ellis, R.J., S.M. Hemmingsen, S.M. van der Vies and S.P. Aldrick. **Molecular chaperons and chloroplasts.** Harden Conference on Plant Molecular Biology, University of London, Wye College, U.K., September, 1987.
14. GrootWassink, J.W.D., A.D. Kolenovsky, J.C. Jain, M.R. Michayluk, K.B. Chatson, D.W. Reed, E.M. Giblin and E.W. Underhill. **Biosynthesis of artificial glucosinolates in Brassica juncea.** Crucifer Improvement Cooperative Workshop, Madison, Wis., October 12-14, 1987.
15. Gummuluru, S., S.L.A. Hobbs and S. Jana. **Physiological characters in drought tolerant and susceptible durum wheat.** Canadian Society of Plant Physiologists/Canadian Society of Plant Molecular Biology Joint Meeting, Kingston, Ontario, June 9-12, 1987.
16. Hemmingsen, S.M. **The Rubisco large subunit binding protein.** RUBISCO 1987 Conference, Tuscon, Arizona, April 20-25, 1987.
17. Hobbs, S.L.A. and J.D. Mahon. **Genetic variability in photosynthesis in peas.** OECD Workshop: Genetics and Physiology of Photosynthesis and Crop Yield, Cambridge, England, July 20-24, 1987.
18. Hogge, L.R., D.W. Reed and E.W. Underhill. **The analysis of desulfoglucosinolate using thermospray LC/MS.** 35th Conference on Mass Spectrometry and Allied Topics, Denver, Colorado, May 24-29, 1987.
19. Kartha, K.K. **Induction of somatic embryos and plantlets from cryopreserved cell cultures of white spruce.** Gordon Research Conference on Plant Cell and Tissue Culture, Plymouth State College, New Hampshire, June, 1987.
20. Kartha, K.K. **Germplasm preservation through tissue culture.** International Congress of Plant Tissue Culture Tropical species, Bogota, Columbia (South America), September 21-25, 1987. (Invited presentation)

21. Kartha, K.K. **Survival of plant cells and organs at ultra-low temperature.** Labatt Symposium on Stress in Living Systems, London, Ontario, October 28-29, 1987. (Invited presentation)
22. Kurz, W.G.W., F. Constabel, U. Eilert and R.T. Tyler. **Elicitor treatment: a method for metabolite production by plant cell cultures in vitro.** 47th International Congress of Pharmaceutical Sciences, Amsterdam, Netherlands, August 31-September 4, 1987.
23. Le Gal, M.F. and S.L.A. Hobbs. **Formation of abnormal nodule meristem in a strain-resistant genotype of Pisum sativum cv. Afghanistan.** 11th North American Rhizobium Conference, Montreal, Quebec, August 9-15, 1987.
24. Mehra, N.S., K.K. Kartha and C. Stushnoff. **Isoenzymes as markers of cultivar identification and genetic variation in tissue culture regenerants of strawberry.** 21st Isoenzyme Conference, St. John, U.S. Virgin Islands, December 1-5, 1987.
25. Pivnick, K. **Chemical host plant location cues used by the diamondback moth, Plutella xylostella.** Annual Meeting of the Entomological Society of Canada, Penticton, B.C., September, 1987.
26. Pivnick, K. **Response to host plant odours by the diamondback moth, Plutella xylostella.** Annual Meeting of the Entomological Society of Saskatchewan, Saskatoon, Saskatchewan, November, 1987.
27. Pivnick, K. **Mating behaviour and the use of a sex pheromone in Stiodiplosis mosellana (Diptera: Cecidomyiidae).** Annual Meeting of the Entomological Society of America, Boston, Mass., December, 1987.
28. Qureshi, J.A. and K.K. Kartha. **Wheat tissue culture: hormonal influences and somaclonal variation.** N.R.C. Wheat/Barley Biotechnology Workshop, P.B.I., Saskatoon, Canada, June 11-12, 1987.
29. Qureshi, J.A. and K.K. Kartha. **In vitro regeneration of wheat: hormonal implications.** XIV International Botanical Congress, Berlin, West Germany, July 24 - August 1, 1987.
30. Saleem, M. and A.J. Cutler. **Cereal tissue culture recalcitrance: Does oxidative stress lead to instability of cereal protoplasts?** N.R.C. Wheat/Barley Biotechnology Workshop, Saskatoon, Canada, June 11-12, 1987.
31. Saleem, M. and A.J. Cutler. **Role of peroxidase in protoplast development: the activity and molecular forms of peroxidase in mitogenic and non-mitogenic protoplasts.** 7th International Protoplast Symposium, Wageningen, The Netherlands, December 6-11, 1987.
32. Saleem, M. and A.J. Cutler. **Uptake of macromolecules by osmotically permeabilized plant protoplasts.** American Society of Plant Physiologists meeting, St. Louis, Mo., July 19-23, 1987.

33. Saleem, M. and A.J. Cutler. **Effect of protoplasting stress on peroxidase and lipoxygenase activities in cereal protoplasts.** American Society of Plant Physiologists meeting, St. Louis, Mo., July 19-23, 1987. *Plant Physiology* 83: 77S.
34. Wang, H.C. and K.N. Kao. **G-banding in Vicia chromosomes.** 32nd Annual Meeting of the Genetics Society of Canada, Saskatoon, Saskatchewan, June 14-18, 1987.

9. Canada's Investment in PBI, 1987-88

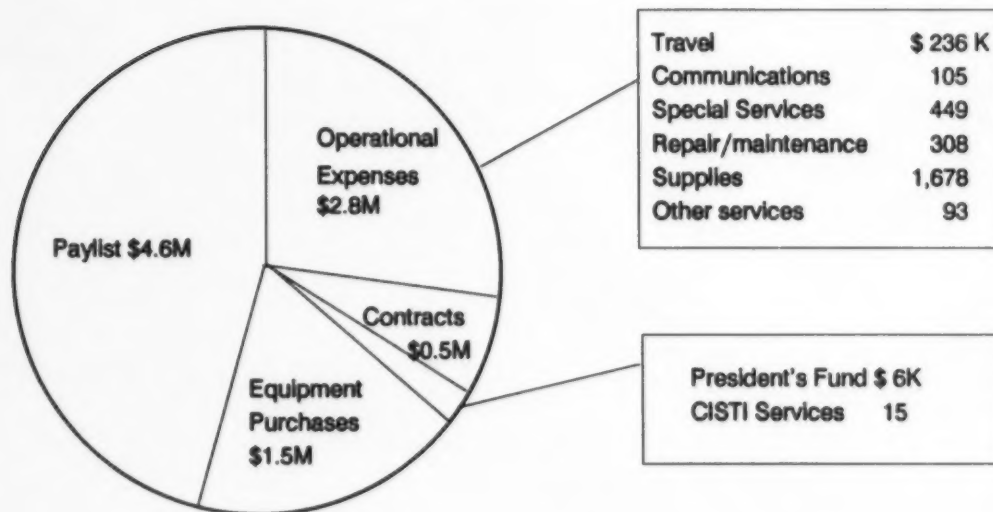
The resources flowing into the Plant Biotechnology Institute increased by nearly 20% in 1987/88. This jump followed three years of steady-state budgets.

Three factors accounted for the increase: arrival of new staff (and therefore start-up of salaries) to fill positions allotted earlier; receipt of awards for excellence and for joint venturing; and the growth of Guest Research contributions. Operational costs also increased but this year not as rapidly as income.

Public expenditures on each project for the year were approximately as shown in the pie graph on the following page; some spending on behalf of more than a single project makes exactitude impossible. Operational spending for PBI overall is broken down in the pie graph below which also refers to public funds.

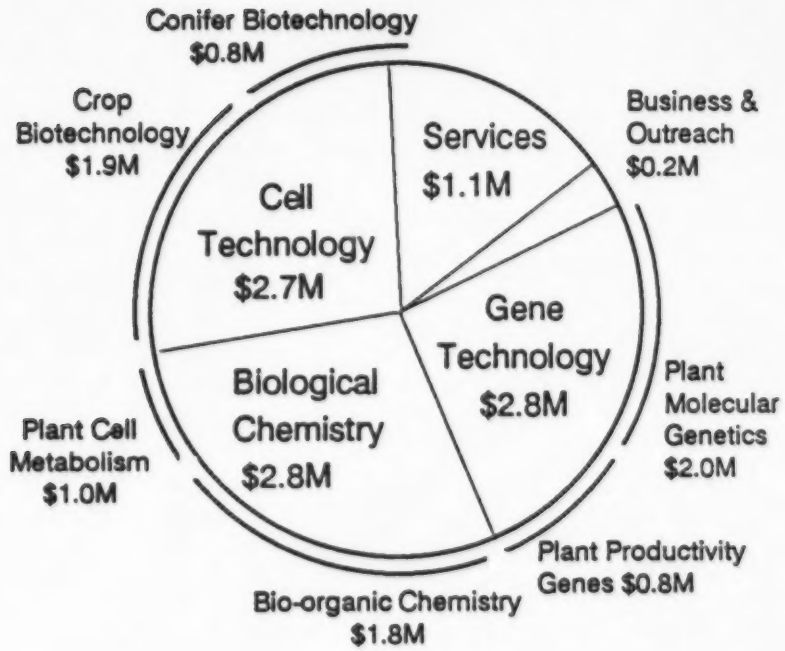
Investment at PBI from external sources continued to increase throughout 1987/88 and at the end of the year stood at its highest level ever. The largest item by far was the salary/stipend cost of sponsoring guest researchers in the Institute.

Expenditures of the Plant
Biotechnology Institute, 1987/88



**Allocation of Funding to
PBI Activities, 1987/88**

Figures include payroll, operations, contract support, equipment



The Year Ahead

The Plant Biotechnology Institute's plans for 1988/89 call for continued efforts in all of the six projects described in this Annual Report. The program thrust will continue to be towards new methods and techniques development, and for outputs of the very highest quality.

Because of a continuing budget crisis at NRC, there will be no new core resources for PBI in 1988/89; indeed the budget may be lower than in 1987/88. The Institute is therefore forced to launch an aggressive search for extramural revenue. Revenue generation features prominently in plans for 1988/89, and the Institute's business unit, created in 1987, will see much activity this year.

The responsibility of the Institute to make its facilities, project action and staff expertise available to Canadian research and development will be recognized through continuance of the Guest Researcher program, the provision of industrial laboratory space, and through a series of bilateral arrangements which PBI has begun to conclude with organizations from coast to coast.

Many of the research activities begun in the early years of the Institute -- in 1984 and 1985 -- are now producing exciting new science and technology, so that despite financial clouds the year 1988/89 will be one of achievement and advance. Some of our results will have major commercial importance as well as scientific impact on plant biotechnology.

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